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POSTERIOR PITUITARY ACTIVITY FROM AN ANATOMICAL STANDPOINT *

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Since representatives of all departments of medicine are now in pursuit of the manifold activities of the pituitary body it may not be untimely to reconsider the histological evidences of the source and nature more particularly of the posterior lobe secretion and its pathway of discharge.

Because of the great interest aroused by the discovery in extracts of the anterior lobe of separable growth-promoting and sex-maturing hormones, attention of late has been focussed chiefly on the activities of that part of the gland. The posterior lobe meanwhile has been largely ignored and the impression has gained ground that it is a relatively unimportant structure. It has been separately extirpated in dogs without recognizable consequences (doubtless because it was not known what effects to look for), and the recently aroused interest in the hypothalamus and its functions has served more than ever to throw it into the shade.

Herring's Hyaline Bodies: After Howell's demonstration (1898) that the previously discovered haemodynamic effects of pituitary extracts were a property of the posterior lobe alone, ten years elapsed before the publication of P. T. Herring's illuminating studies [9,10] of this particular subdivision of the gland. He described, in the pars nervosa of cats, what he called hyaline bodies,† which were looked upon as the secretory product of the pars intermedia.

* Received for publication June 9, 1933.

† It was possibly an unfortunate designation as the term "hyaline" suggests to pathologists a form of tissue degeneration. Others speak of the same substance as "colloid."

These bodies or masses made their way through the nervous tissue, along what were taken to be lymph spaces, in the direction of the infundibular cavity into which some of them appeared to pass.

This conception of posterior lobe secretion, though accepted wholly or in part by a few observers (*e.g.*, Cushing and Goetsch,[7] da Costa,[5] Remy Collin,[4] Biedl[3]), has been looked upon in most quarters as wholly fantastic. Attempts on the part of many to establish the actual presence of posterior lobe hormone in the cerebrospinal fluid have led to contradictory results. Possibly the two most convincing recent observations in support of this view have been: (1) by Karplus and Peczenik[11] (1930), who found a pressor substance in the cisternal fluid only after electrical stimulation of the tuber; and (2) by Zondek and Krohn[22] (1932), who have shown that a melanophore-expanding substance (*Intermedin*) proves not only to be abundant in extracts of the pars intermedia, of stalk and of tuber, but is also demonstrable in small amounts in the fluid of the third ventricle. In the cerebrospinal fluid elsewhere it is not detectable.

Important information regarding the tubulo-infundibular apparatus has meanwhile been secured from two sources. One of them has been the demonstration (1925), independently by Greving[8] and by Pines,[14] of the rich network of unmyelinated fibres from supra-optic and tuberal nuclei which arborize in the posterior lobe, thereby confirming Tello's observation[20] (1912) on the human gland of what Ramón y Cajal[18] had originally (1894) described in the mouse. The other has been the description by Popa and Fielding[15, 16, 17] (1930–1933) and Basir[2] (1932) of an "hypophysio-portal" circulation whose main venous trunks, having gathered blood from the sinusoids of the pars distalis, ascend in the pars tuberalis to break up in a vascular capillary plexus in the tuber cinereum (*cf.* Figs. 2–12). It is assumed that these ascending veins, which were independently described by Pietsch[13] (1930), serve to carry the products of pars anterior secretion, colloidal masses of which may occasionally be demonstrated[17] in the lumina of the vessels.

There thus appear to be two routes, both probably under nervous control, whereby the products of pituitary secretion are transported to the tuberal and possibly to other nuclei of the anterior hypothalamus: (1) from pars anterior by way of the "hypophysio-portal" vessels; and (2) from pars intermedia by way of the tissue

spaces in the pars nervosa. Though something will be said of the former route of secretion, it is with the latter that this paper will chiefly deal.

Among those who have described the course of the hyaline bodies through the pars nervosa and who believe that it has a stimulatory effect on the tuberal nuclei, only a few (Herring in the cat, Cushing and Goetsch in the dog, and Remy Collin in birds) appear to have noted that hyaline masses may actually be seen squeezing their way between the ependymal cells to enter the infundibular cavity. Observations of this kind are fortuitous because there is no certain method of preserving the secretory product in the tissues. For owing to the ready solubility of the substance it is usually dissolved out in the process of tissue fixation, leaving only an occasional hyalinoid mass still retained in the empty spaces in the open glial meshwork. That these empty tissue spaces which comprise the greater part of pars nervosa, stalk, tuber and infundibular ependyma serve as a tell-tale of the former presence of the secretory product, an effort will be made herein to show.

The Source of the Posterior Lobe Hormone: Preparations of the active principle of the posterior lobe are customarily made from the glands of animals in which there is a residual cleft. Hence, the extracts are not solely of pars nervosa, as generally assumed, but of pars intermedia as well. It is scarcely conceivable that the neural core of the lobe is capable independently of elaborating a hormone, which obviously must be produced by its inseparable epithelial investment. The two are in intimate functional contact, for the infundibular projection represents the only portion of the central nervous system that remains unprotected by an essentially impermeable pial covering.

Because of the absence of a distinct cleft in the glands of man and higher apes certain writers disclaim the existence in them of a separately recognizable pars intermedia. This, however, is an academic point of view that has been well answered by Rasmussen [19] (1930), who believes with others that the elements comprising the pars intermedia are clearly distinguishable. Even under normal conditions, when the lobe is relatively inactive, the chromophobe mother cells of the juxtaneural investment tend to arrange themselves into colloid-holding vesiculi, from which an occasional ripened cell may be seen invading the adjacent neural tissue.

In the process of their ripening the cells of the pars intermedia invariably become transformed into basophilic elements which in their mature stage are indistinguishable from the ripened basophiles of the pars distalis. The cells may occasionally be seen in the process of disgorging their cytoplasm in apocrine fashion, but more often the entire cell body is cast off in the manner of a holocrine secretion (*cf.* Fig. 14). The highly vacuolated elements thus discharged soon lose their tinctorial affinity and each separate mass as it becomes "hyalinized" in its passage into the lobe may long retain the recognizable ghost of the swollen nucleus (*cf.* Figs. 15–16). These so-called hyaline bodies of Herring are certainly not the degenerated end-bulbs of nerves as some writers, under the influence of Tello's paper, have come to believe.

Basophilic Activation of Posterior Lobe: When the normal activity of the pars intermedia is exaggerated in certain pathological states there occurs a marked hyperplasia of the basophilic elements. Not only do they discharge their secretory product into the relics of the original cleft, which can be partly reestablished thereby, but the ripened elements wander deeply into the neural tissue after the manner of a malignant epithelial invasion, to which the process first described by Thom [21] (1901) has often been likened. While this infiltrative tendency has been observed by many, it has been commonly supposed to be a peculiarity of the glands of aged persons (more particularly of those victimized by arteriosclerosis and renal disease) and to have no functional significance.

These infiltrating elements, which are apparently unrelated to the tubulo-racemose glands recently redescribed by Lewis and Lee [12] (1927), are accompanied by a great increase in the hyalinoid secretory product that gives to the lobe, stalk and tuber its "juicy" appearance to the naked eye. And when sections are cut and the pars nervosa is found to be composed of a widely opened mesh (from which the hyalin has been largely dissolved out), it is usually described as representing an "oedematous" condition of the tuberoinfundibular structures.

As must have been observed by all who have studied serial sections through this region in whatever plane they happen to have been cut, this familiar "oedematous" or open appearance of the tissue is sharply confined to pars nervosa, stalk, tuber cinereum and neighbourhood of supra-optic and paraventricular nuclei. Attention, however,

does not seem to have been specifically drawn to the peculiar fact that the ependymal cells lining the lower infundibular cavity are invariably broken up by this so-called "oedema," whereas the ependyma of adjacent parts of the ventricle remains more or less intact.

The impression is inescapable from this appearance that something (the secretion in all probability) is forcing the cells apart and being extruded into the ventricle. At the tip of the infundibular cavity there may be no trace whatsoever of the ependymal lining (*cf.* Fig. 17) and patches of it begin to appear only as one passes upward (*cf.* Fig. 18). Not infrequently one sees the more or less intact ependyma lifted into a bleb that has the appearance of rupturing into the ventricle (*cf.* Figs. 19–20), after which a new cuticular layer of cells reforms. Even when the lining of the infundibular cavity is found to be reasonably intact, should the cells happen to be cut lengthwise in the direction of their long tails, the cell bodies here and there seem to be irregularly separated, as though mechanically forced apart. This broken-up appearance of the tuberoinfundibular ependyma must have been observed by many and likely enough been ascribed to an artifact from some fault in tissue fixation or section cutting.

Posterior Lobe Basophilia in Disease: Moderate degrees of basophilic infiltration of the pars nervosa have often been described with no suggestion made, or at least no emphasis laid, on the possibility that it represented a physiological activation of the neurohypophysis. And while it is not the purpose of this communication to do more than point out what are looked upon as histological indications of posterior lobe activity, the reasons for bringing the matter up anew at this time may briefly be given.

A long known polyglandular disorder has been shown to be caused by an actively secreting basophilic adenoma of the pars distalis. In the study by serial sections of the pituitary body and interbrain of a fatal case of this disorder (as told in another place [6]), a most extensive infiltration of basophilic elements from the pars intermedia was disclosed. Since the clinical disorder, among other symptoms, is characterized by hypertension, glycosuria and adiposity, which suggest a posterior rather than anterior lobe effect, it was assumed that the cellular invasion of the pars nervosa might well have been the causative factor in these symptoms and might therefore represent a physiological hyperactivation of the posterior lobe.

Aware that Anselmino and his co-workers [1] had found in the blood of patients suffering from the hypertensive toxaemias of pregnancy (eclampsia) a substance giving the reactions of posterior lobe extracts, also that Irvine Page (personal communication) had found a similar or the same substance in the blood of certain patients with so-called essential hypertension, as opportunity has arisen studies have been made of the glands secured from a few fatal examples of these two disorders.

These glands have shown, in somewhat less degree, the same massive basophilia of the pars nervosa seen in the aforementioned polyglandular disorder. It is assumed, therefore, that the process in all probability *does* indicate an hyperactivation of the posterior lobe and consequently represents the pathological basis of these hypertensive maladies. It is perhaps not overventuresome to predict that in some of these hypertensive states the posterior lobe principle may prove to be detectable in the fluid of the third ventricle as well as in the blood stream.

FIG. 1. The original block of tissue (natural size).

Illustrative Material: The accompanying photograph (Fig. 1) shows the original block of tissue from a fatal case of essential hypertension.* It is viewed from behind and shows the small, somewhat cupped, gland with prominent posterior lobe, the stalk and tuber being distinctly "juicy" in appearance. After fixation in formalin the entire block was cut serially from below upward on the horizontal plane in 8 micron sections, every tenth section having been stained with haematoxylin and eosin† and numbered accordingly.

* For this particular specimen I am indebted to Dr. George Hass of the pathological department of the Peter Bent Brigham Hospital.

† The preferential method (1) of fixing, (2) of cutting and (3) of staining the pituitary body naturally differs with what may be the object of the study. Da Costa,[5] for example, states that the hyaline bodies are better preserved in small tissue fragments fixed in Flemming's solution, with which we have had no experience. For our particular purposes it is undesirable that the gland should be cut in parts before fixation, as is commonly done. Hence, owing to its greater penetrability, formalin has distinct advantages over Regaud's, Zenker's or Kolatchew's fluid.

For our purposes also, serial sections in the horizontal plane prove to be preferable to coronal or sagittal sections as they give better topographical orientation, particularly should the block include the tuberal region as well. (*Continued on opposite page.*)

Photomicrographs of certain selected sections from this single case will suffice, it is hoped, to bring out the principal points relating to posterior lobe activity, which it is the purpose of this communication to reemphasize. For orientation all sections should be regarded as being seen from above, the anterior structures lying at the top of the print, the posterior structures at the bottom. Figures 2 to 12 have been chosen for the purpose of tracing on these horizontal sections the "hypophysio-portal" circulation from pars anterior to tuber cinereum. Figures 13 to 20 serve to show the formation (from the inwandering basophilic elements of the pars intermedia) of the secretory product and its upward passage through pars nervosa and stalk toward the ventricle. The legends facing these illustrations (to be found at the end of the text) will render it superfluous to redescribe them here.

Summary

In all the recent attention paid to the pituitary body and its functions, the posterior lobe has been much neglected because of the greater temporary interest in the hormones of the pars anterior and the newly discovered activities of the interbrain. It nevertheless has an active principle or principles capable among other properties (1) of raising blood pressure, (2) of contracting smooth muscle, (3) of causing hyperglycaemia, (4) of expanding cutaneous melanophores, and (5) of diminishing renal secretion.

Its secretory product is unmistakably derived from the investing pars intermedia, whose cells become basophilic when ripened. Doubtless under nervous impulses from hypothalamic nuclei these cells, by a form of holocrine secretion, are cast off, invade the pars nervosa and become transformed into "hyaline bodies," which as Herring first showed in lower animals make their way through the loose neural spaces of pars nervosa in the direction of the infundibular cavity. They may, in favourable preparations from the cat and

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Simple haematoxylin and eosin stains give perfectly satisfactory cellular differentiation when the solutions are properly used. While many stains, like Mann's eosin-methyl blue and Mallory's aniline-blue orange G, give a more brilliant colour contrast, this is often at the expense of a clear picture of the cellular morphology. The specific stains for the granules, such as Bailey's acid-violet eosin or ethyl-violet orange G, after Regaud fixation, or Severinghaus' acid-fuchsin methyl-green acid-violet stain after Kolatchew fixation, for the reasons given are inapplicable to the serial study of a large block of tissue.

dog, even be seen to extrude themselves into the ventricle between the ependymal cells.

In certain conditions of disease characterized notably by hypertension, but also by other symptoms suggestive of known physiological effects of posterior lobe extracts, the normal cellular activity of the pars intermedia becomes greatly exaggerated. This is shown by a marked hyperplasia of the basophilic elements that penetrate far into the lobe. Under these circumstances not only is the visible secretory product (hyalin) greatly increased in amount over what is customarily seen in supposedly normal glands, but the cuticular ependyma of the lower third ventricle has a highly broken up appearance.

An extreme degree of cellular hyperactivation of the posterior lobe was first observed in the pituitary body of a fatal case of what is known as pituitary basophilism associated with a basophilic adenoma of the pars distalis. Since then a similar condition has also been found in cases of eclampsia and of so-called essential hypertension. In both of these latter states there has been found in the blood stream a substance indistinguishable in its effects from posterior lobe extract, but not detectable in the blood under normal conditions.

The conclusion is drawn that the infiltrative basophilia of the pars nervosa is an expression of functional hyperactivation of the posterior lobe and may be taken to represent the pathological basis of these hypertensive disorders. What particular form of neurohumoral stimulus primarily incites the neurohypophysial basophilia remains to be determined.

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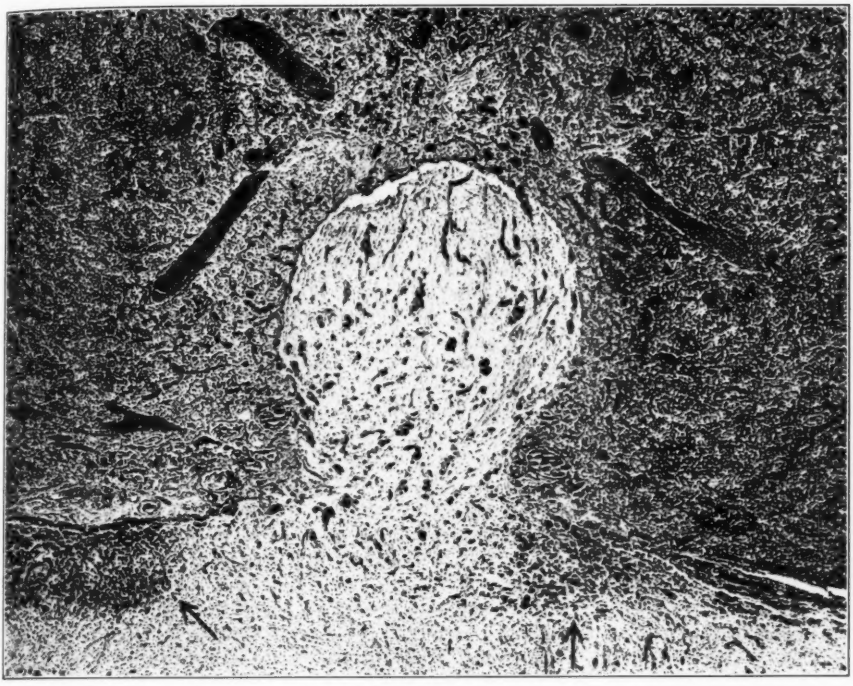
DESCRIPTION OF PLATES

PLATE 75

FIG. 2. (Section No. 40: mag. × 30.) This section transects the upper part of the gland where chiefly acidophilic elements abound. Three large collecting sinusoids can be seen in the pars anterior (above) passing in the general direction of the root of the stalk which appears in the centre of the field. Even at this high level the infiltration of the pars nervosa by basophilic elements (arrows) from pars intermedia is abundant (*cf.* Fig. 13).

FIG. 3. (Section No. 52: mag. × 30.) This section passes through the base of the emerging free stalk and shows the anteriorly placed situation of the pars tuberalis through which pass the large ascending "hypophysio-portal" venous trunks. At the upper left (arrow) lies a small subarachnoid clot from an agonal extravasation. Numerous, minute, capillary haemorrhages are present throughout the pars nervosa, not visible at this low magnification.



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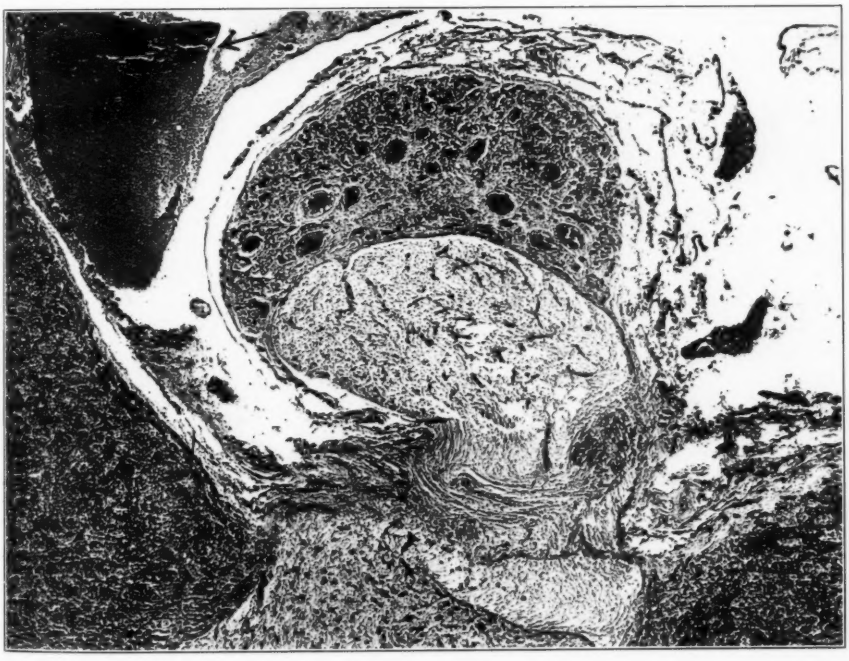

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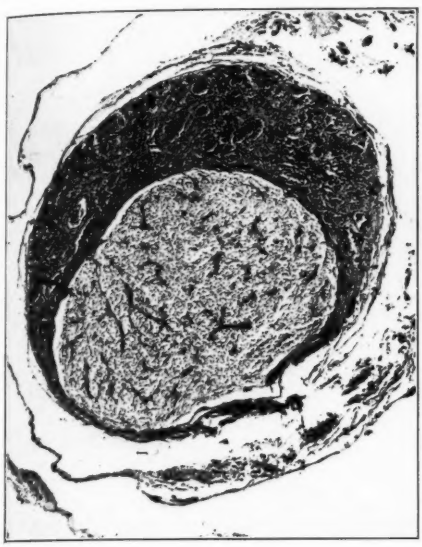
Cushing Posterior Pituitary Activity

Plate 76

Fig. 4. (Section No. 61: mag. × 30.) At this level the stalk is entirely free and beginning to be completely enveloped by the cuff of pars tuberalis. This still holds the many ascending portal trunks, which, as Popa and Fielding have shown, are without cross anastomoses.

Fig. 5. (Section No. 85: mag. × 30.) This shows the tendency for the cuff of pars tuberalis to swing toward the sides and back of the stalk as the tuberal enlargement is approached.

Fig. 6. (Section No. 115: mag. × 30.) Here the stalk is enlarging into the tuber at the near proximity to the chiasm shown in the upper part of the field. The "hypophysio-portal" vessels are beginning to leave the cuff of pars tuberalis to plunge into the nervous tissue, forming glomerulus-like tufts (the "secondary capillary net" of Popa and Fielding) enveloped by comparatively dense glial sheaths (*cf.* Fig. 12).



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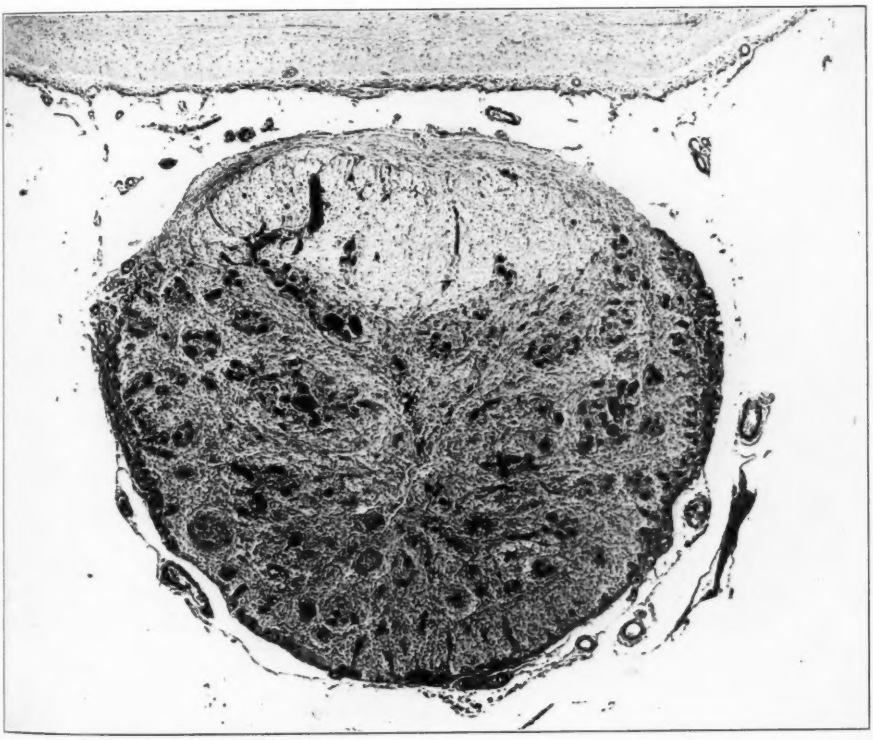

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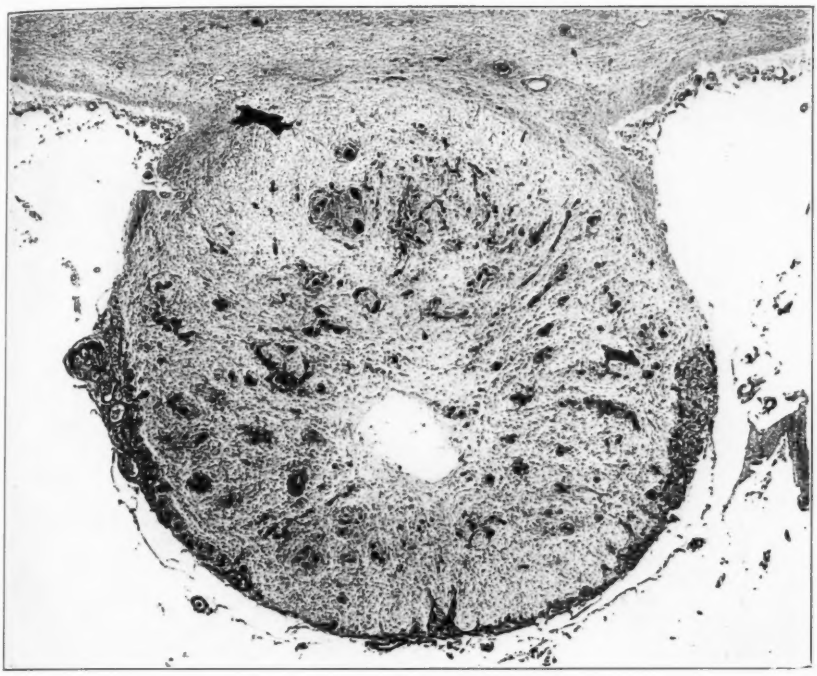
Plate 77

Fig. 7. (Section No. 124: mag. × 30.) Here the tuber cinereum is fused with the posterior margin of the chiasm, narrow residuals of the pars tuberalis being still evident on its posterior and lateral surface. The tip of the infundibular cavity with its disrupted ependyma (*cf.* Fig. 17) is apparent in the lower centre of the field.

Fig. 8. (Section No. 133: mag. × 30.) The terminal capillary bed of "hypophysio-portal" vessels has here largely swung around to lie anterior to the infundibular cavity. The cuticular ependyma of the ventricle is still missing on the anterior side, but posteriorly the cavity begins to show an intact ependymal lining. Traces of the tuberal cuff are still discernible on the postero-lateral surface of the tuber.



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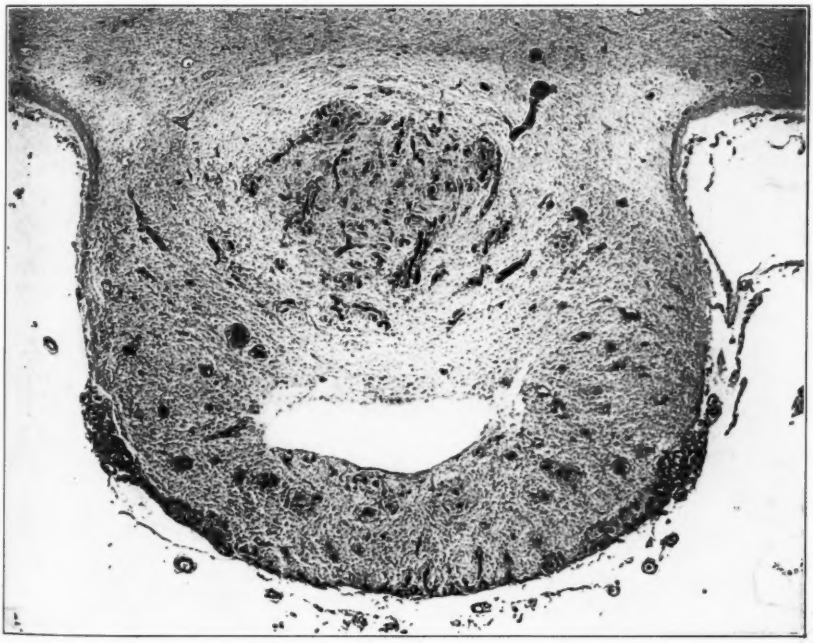

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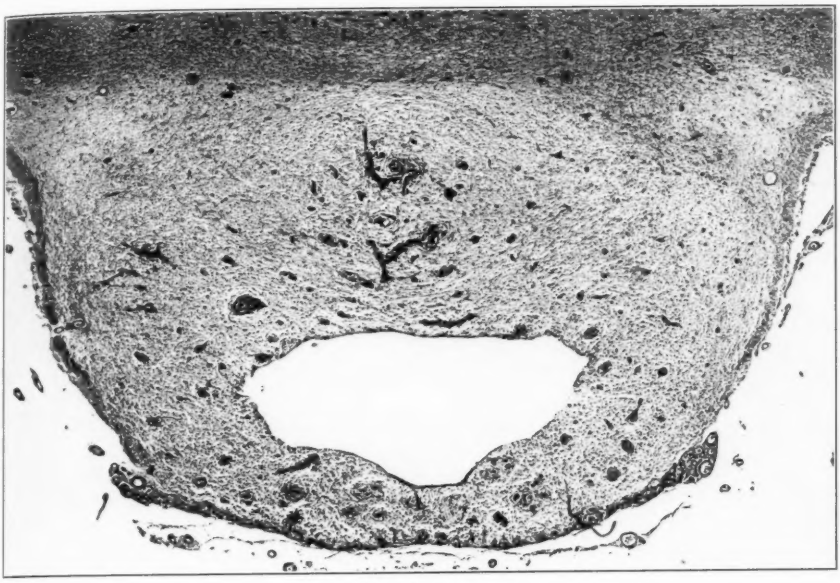
Plate 78

Fig. 9. (Section No. 142: mag. × 30.) The tuber cinereum here is flattened out against the posterior surface of the chiasm. The loose texture of the tuberal tissue is clearly distinguished by its light shade even at this low power. The cuticular ependyma of the widening cavity is fairly intact, except at its lateral angles. Traces of the terminal capillary plexus of the portal system surrounded by its glial sleeve are still to be seen between ventricle and chiasm.

Fig. 10. Showing (mag. × 175) homogeneous and refractile masses of colloid (?) in two of the ascending hypophysio-portal vessels of upper stalk (arrows). From a case of "pituitary basophilism" due to an adenoma. Colloidal masses in this case are also to be seen in the capillaries of the pars nervosa.



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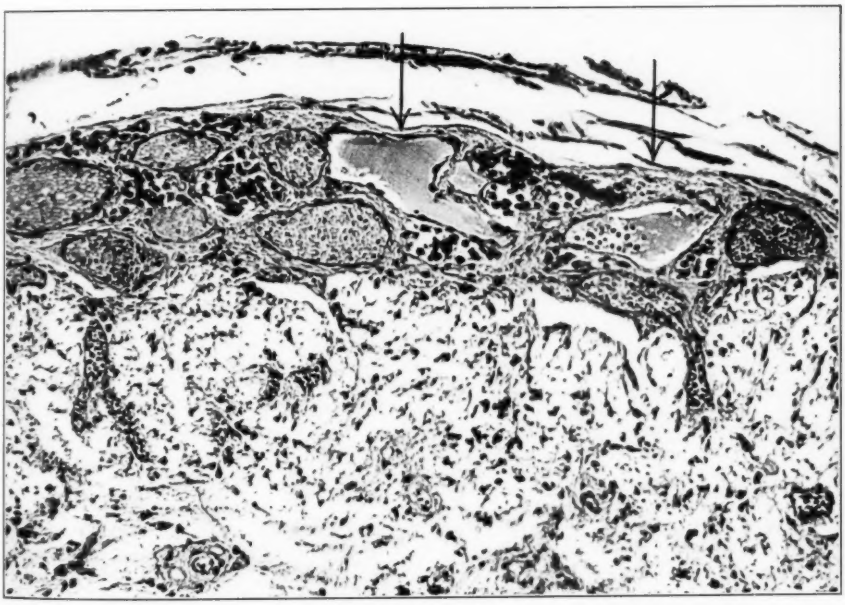

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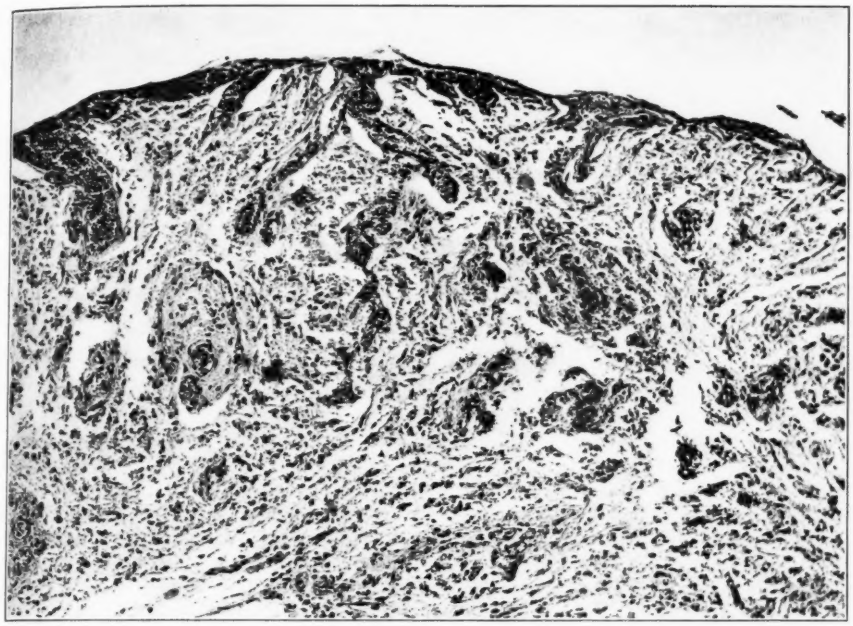
PLATE 79

FIG. 11. Showing (mag. × 100) at level of tuberal enlargement of upper stalk how the large ascending portal trunks break up into smaller vessels which leave the residual of the pars tuberalis to plunge into the tuberal tissue forming the secondary capillary net.

FIG. 12. (Section No. 119: mag. × 150.) A glomerulus-like tuft of capillary vessels with glial sheath characteristic of the vessels composing the secondary net.



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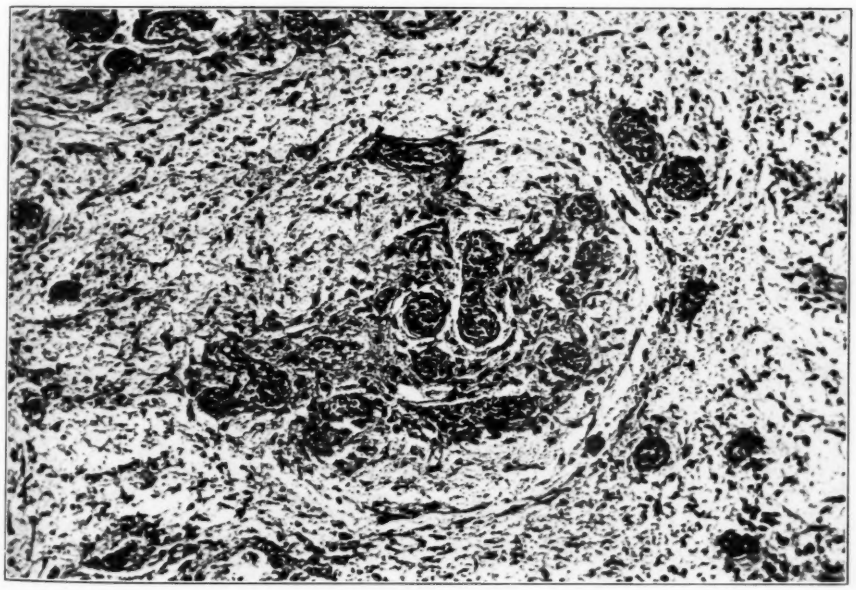

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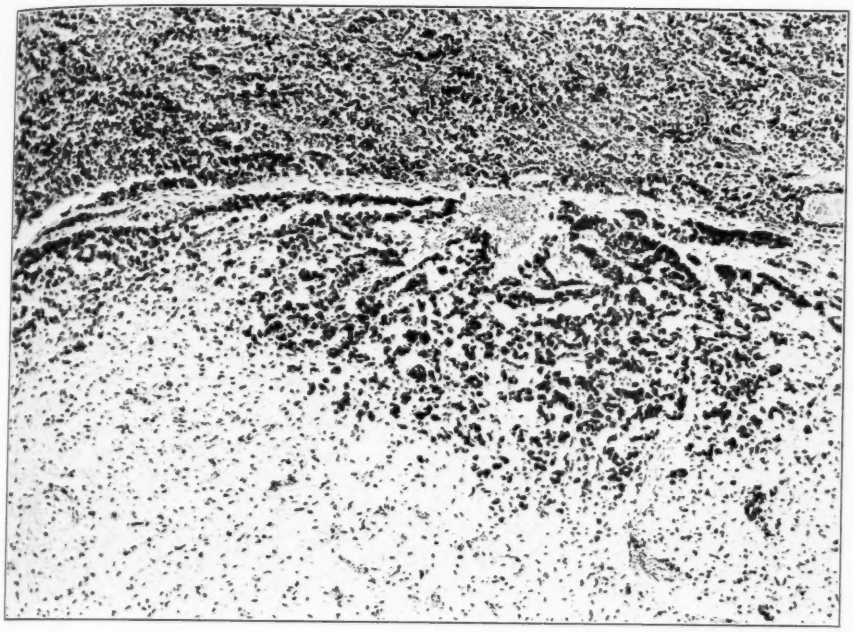
Plate 80

Fig. 13. (Section No. 34: mag. × 80.) This section passes approximately through the centre of the gland and transects the body of the posterior lobe. It shows the character of the basophilic invasion of the pars nervosa by pars intermedia cells. This invasion occurs in varying degrees of depth from almost the entire juxtaneural epithelial surface.

Fig. 14. Showing (mag. × 850) the masses of secretory holocrine product lying between the tongues of invading and fully ripened basophilic elements. The cast-off cells are still highly granular in appearance but have largely lost their tinctorial affinity to haematoxylin (they stain a reddish brown with Mallory's phosphotungstic acid haematoxylin). In the granular protoplasmic mass of many of the discharged cells the trace of the greatly swollen nucleus is often discernible (*cf.* Fig. 15).



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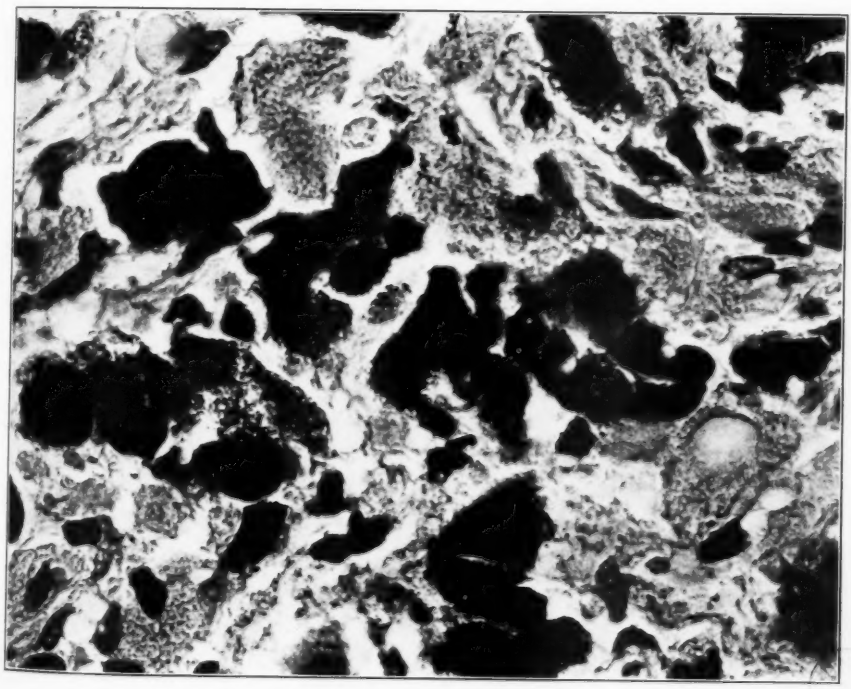

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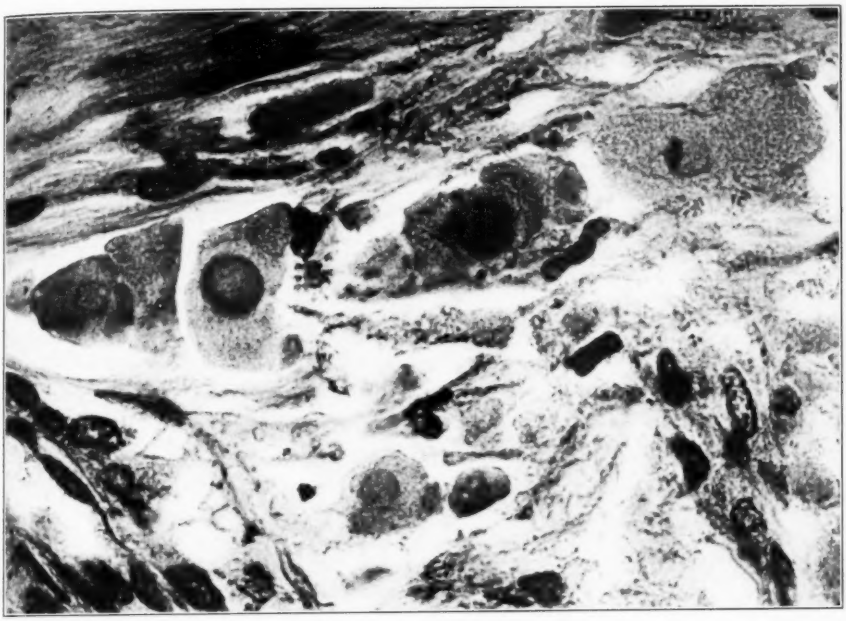
Plate 81

Fig. 15. To show masses of cast-off holocrine secretion making their way upward through the interstices of the pars nervosa. Ghosts of swollen nuclei still discernible in the secretory masses which become increasingly homogeneous (hyalinized) in their passage through the lobe (mag. × 850).

Fig. 16. Occasional hyaline masses are even to be found trapped in the loose tissue spaces of the tuber. It is assumed from the appearance of the tuberal tissue that the open mesh was once full of the secretory product that has been dissolved out in the process of fixation (mag. × 300).



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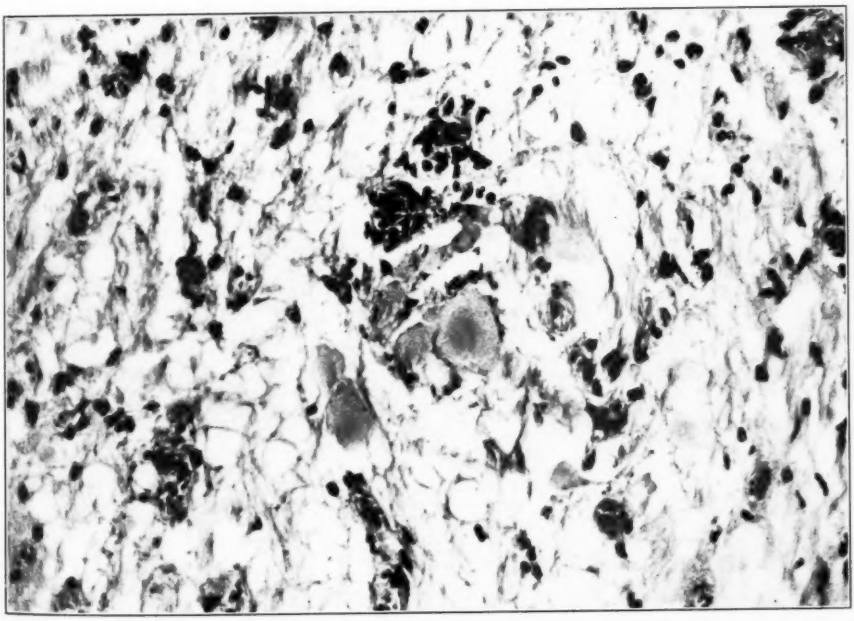
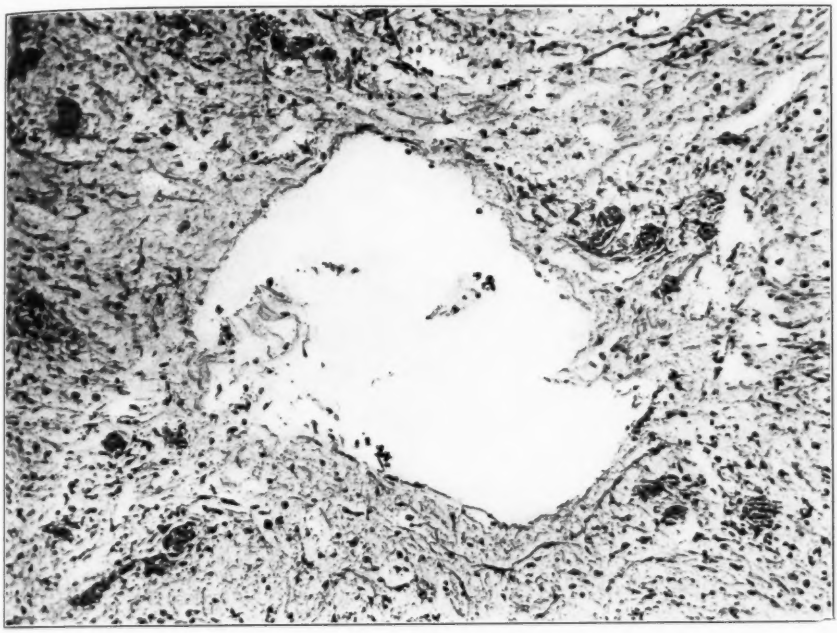


16

Plate 82

Fig. 17. (Section No. 124: mag. × 125.) To show at higher magnification a cross-section of the tip of the infundibular cavity (*cf.* Fig. 7). Note the broken-up appearance of the ependymal lining which is invariably present in this region. No cuticular layer is here discernible.

Fig. 18. (Section No. 131: mag. × 125.) To show tip of infundibular cavity slightly higher up than in Fig. 17 (above). Here two loops of intact cuticular ependyma (arrows) are present with an intervening break in the lining. Otherwise one would hardly know that the open spaces represent the ventricular cavity.



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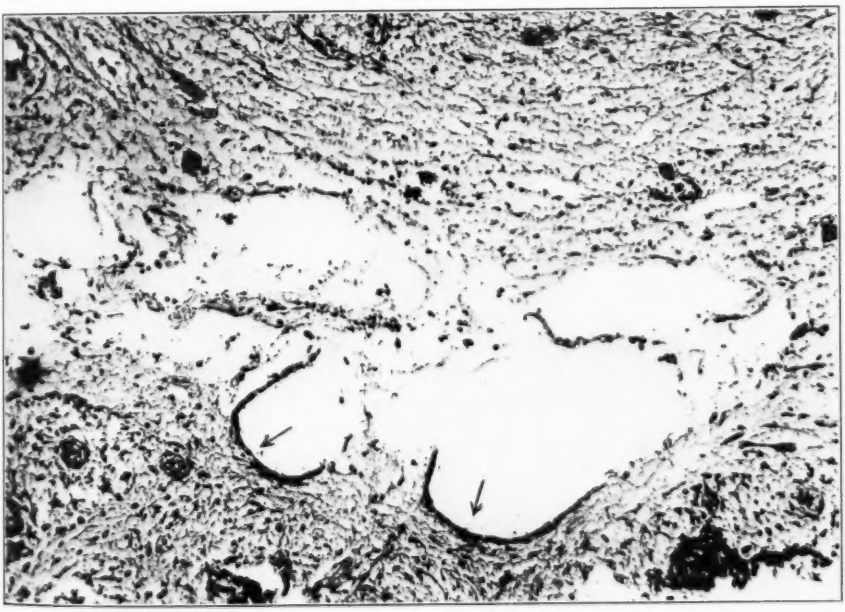

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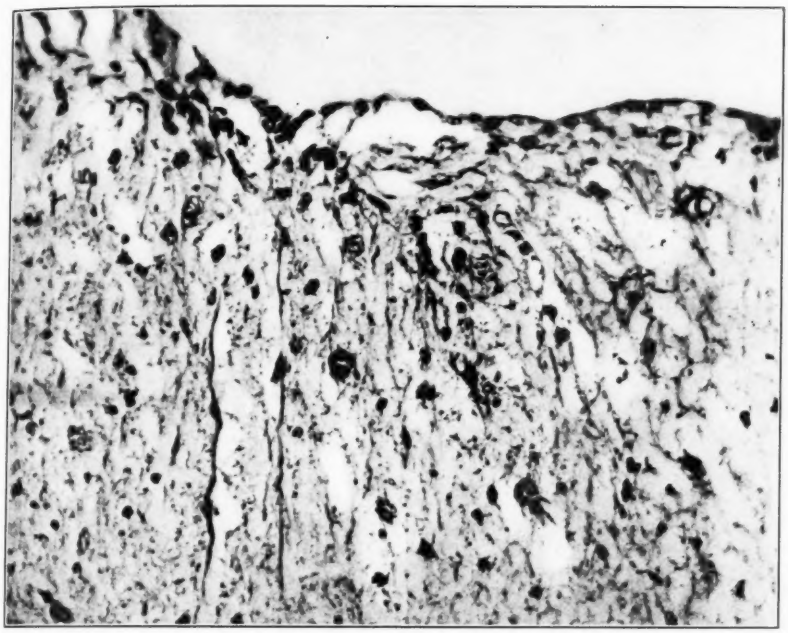
Plate 83

Fig. 19. (Section No. 151: mag. × 300.) To show a characteristic bleb forming under the still intact cuticle. As these subependymal blebs form, bulge into the ventricle and rupture, a new line of ependymal cells reforms below the protrusion and comes to take the place of the original surface layer.

Fig. 20. A more highly advanced stage (mag. × 300) of what is shown in Fig. 19, indicating the rupture and escape of something (presumably secretion) into the ventricle. Note the tendency of new ependymal bodies to reassemble on the neural side of the bleb.



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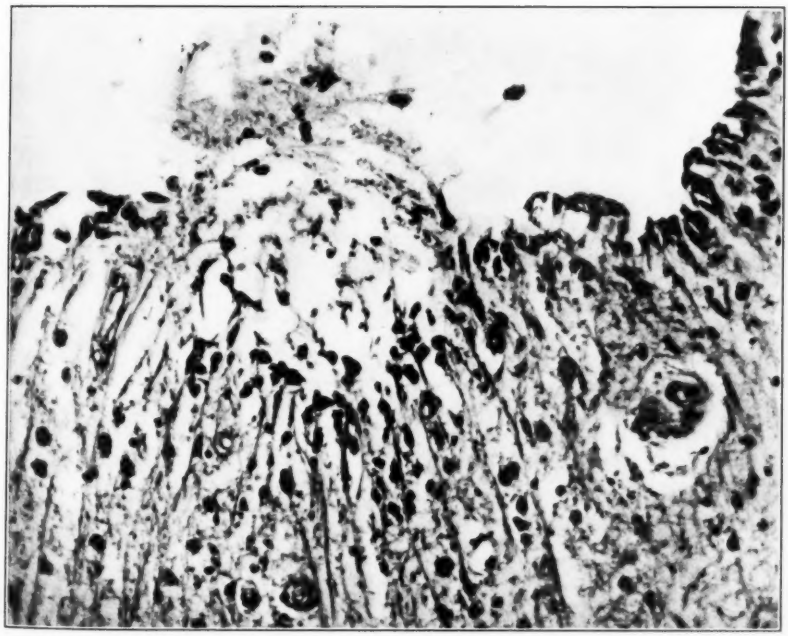

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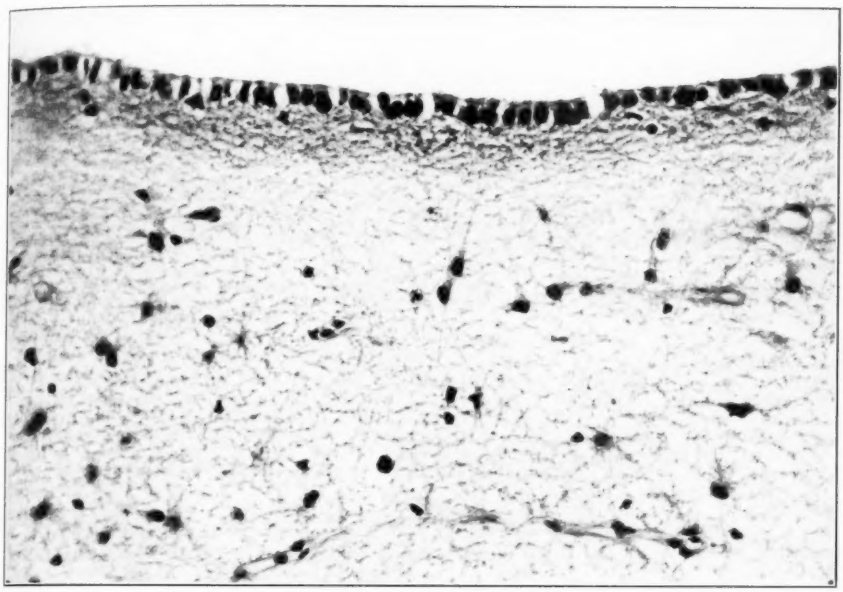
PLATE 84

FIG. 21. In contrast with Figs. 19 and 20, here is shown (mag. × 300) the fairly intact ependymal lining of the lateral and dorsal aspects of the anterior third ventricle. That this view is taken from the tuberal region is shown by the characteristic open network of the subependymal tissue.

FIG. 22. Here is shown (mag. × 300) the loose (oedematous) character of the tissue in the region of both paraventricular and supraoptic nuclei, presumably opened out by stimulatory secretion. The adjacent neural tissue elsewhere is normally compact.



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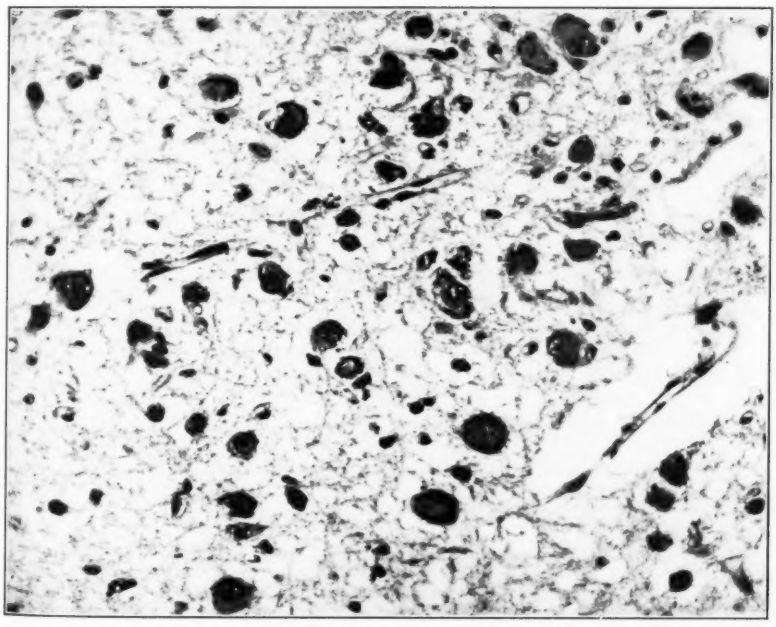

22

A CASE OF MALFORMATION ASSUMED TO BE TRUE HERMAPHRODITISM *

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The subject of the malformation to be described is a middle-aged woman, somewhat slender but of great physical beauty and charm. Her complexion is so fine that she needs no rouge; her hair, which has never been bobbed, is beautiful, long and fine; her bust is of rather more than average size; her voice is a distinct soprano, and she has been happily married to a member of the medical profession for years. At no time has she ever shown any suggestion of any endocrine abnormality. She menstruates regularly every three weeks. In her early married life she was once told by a physician that it was improbable that she would ever have a child, but the patient remembers no reason for this statement and the matter was temporarily forgotten until later when she believed herself to be pregnant. She missed one period, had a little "stomach distress" which lasted a week or two, then, following a typhoid fever inoculation, again menstruated, the clots being a little more numerous and larger than normal. There has never been any other suspicion of pregnancy, although no precautions for its prevention have been taken.

Some years ago, in preparation for an operation for the removal of a urethral caruncle, the external genitalia were examined and no malformations of any kind detected. The clitoris was not enlarged. At that time a routine vaginal examination was made. Something "seemed to be wrong" and the surgeon jumped to the hasty conclusion that a panhysterectomy had been performed sometime previously and the vagina shortened. It was later learned that she had never before been operated upon.

In the early part of 1931 the patient had a fall and shortly afterward discovered a swelling in the groin. As it soon disappeared, it

* For the specimen, data and permission to publish this interesting case, I am indebted to Dr. Jonathan Forman and to Drs. J. F. and Hugh A. Baldwin, to whom I here tender my appreciation of their generosity and extend my thanks.

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was supposed to have resulted from extravasation. Later, however, it reappeared and upon examination was found to be a femoral hernia. At a convenient time an operation for the correction of the abnormality was performed.

Notes made at the time of the operation describe it as follows: "No hernial protrusion was seen or felt, but the finger could be introduced into the femoral canal. The usual incision was made, the sac easily found, freed, pulled down and opened, exposing what was thought to be a sliding hernia. On pulling this down a body whose size and shape resembled an ovary was found, detached and removed, after which the sac was freed high up, ligated, and the opening closed with two chromicized catgut sutures in the usual manner."

"Examination of the tissue failed to show anything that could be identified as ovarian tissue. One part suggested possible malignancy."

It was because of this suspicion of malignancy that sections of some of the tissues were sent to me, and because of my report that the case was not one of tumor but of hermaphroditism that the whole matter was placed in my hands for study and publication.

As frequently happens when the importance of a specimen is not at first realized, the tissues removed at the operation were badly mistreated. They had been cut through in various directions and then crowded into a very small container of fixative in which they hardened in positions making it impossible to determine accurately the relation of the parts. It has not even been possible to discover which was the top or bottom of the whole or what its attachments may have been. In an endeavor to find out exactly what the relations had been the following answer was received: "After opening the sac of the femoral hernia the mass appeared and, as originally stated, was at first suspected to be an ovary. It was intraperitoneal, that is, if you call an ovary intraperitoneal, and seemed to be attached by a sort of pedicle to the side of, or just within the femoral canal. It was cut off at that point and the base whipped together. The rest of the operation was completed in the usual manner, removing the sac and obliterating the opening with chromicized catgut. No drawing that I could make would help in explaining its location."

Although at first it seemed impossible to learn much from the examination of the gross material, after repeated careful study of

all the parts and fitting them together as well as I was able, Mr. Erwin F. Faber, artist to the medical laboratories of the University of Pennsylvania, succeeded in making the diagrammatic sketch shown in Figure 1, which represents, at about the natural size, a fair approximation of the original arrangement.

At the extreme upper part of the sketch at *a* is a portion of a broad tubular canal, at first glance not unlike a piece of intestine, about the thickness of the ileum and more or less rugose. Microscopic examination, however, revealed that it was serous membrane, and it is probably the excised part of the hernial sac.

Immediately below and attached to it at *b* is an ill-defined, dark colored, fibrillar tissue mass, the nature of which was very puzzling. Histologically it is composed almost exclusively of unstriated muscular tissue with many blood vessels and many narrow epithelial tubules.

Below it at *c* is a sharply defined ovoid body, to which is loosely attached by serous membrane an elongated tubular structure free at the end toward the left and disappearing in the muscular mass to the right, above and behind. The ovoid body was at first supposed by the surgeons to be a prolapsed ovary, and the tubular structure its Fallopian tube, but when the body was incised it at once became evident that it was not an ovary, and they came to the erroneous conclusion that it was a tumor of some kind. At *e* a tiny pedunculated cyst is attached to the free end of the supposed Fallopian tube, while at *g* a larger cyst appears to spring by a long pedicle from the capsule of the body at *c*.

Careful examination of the body *c* shows it to be covered with serous membrane beneath which is a tough fibrous investiture, and to be made up of a uniform substance of firm consistence and of a cream (?) color.

Upon microscopic examination it at once became clear that the body was neither an ovary nor a tumor but a testis, for it consists entirely of closely approximated epithelial tubules. This being the case, the attached tubular structure would not be an oviduct but might be an epididymis. The little cyst *e* might arise from the organ of Giraldes, and the larger one *g* be the stalked hydatid. Assuming all this to be correct it only remains to point out the homology between the fibromuscular tissue and the spermatic cord to make all of the conclusions consistent.

The confirmation of each of the parts by microscopic examination presented little difficulty.

The Testis: As has been said, this organ is surrounded by serous membrane, the tunica vaginalis, and by a tough fibrous investiture, the tunica albuginea. There is no division into lobules and the usual perfectly regular glandular pattern prevails (Fig. 2). The organ is immature and non-functional. Each tubule consists of a well developed basement membrane, layers of doubtful cells (Sertoli cells?) of semicolumnar form attached to the basement membrane and directed toward the centers of the tubules, whose lumens are vague. It is a question whether the structures should be regarded as genital cords with germinal cells, or as seminiferous tubules. By whatever names they are called they are certainly the homologues of the latter.

The Epididymis: There is neither a distinct head (globus major) nor any definite tail (globus minor) and it is evident that the organ is hypoplastic. The position of the stalked hydatid *g* suggests where the globus major ought to be. Sections of the tissue taken at *d*, shown in Figure 4, show the presence of a spermatic duct with collateral tubules. To determine the histological details more accurately blocks of tissue were cut out between the parallel lines at Figure 1 *d* and *f*, and microscopic sections made. At the center of the broadest part of the organ *d* an epithelial-lined, widely dilated canal is found. It is surrounded by a muscular investiture of varying thickness penetrated by many good sized vessels such as might represent the pampiniform plexus. The dilatation of the canal seems to be caused by the collection of a fluid rich in some colloid that has been condensed by the fixative, and holding to the cellular lining during its transformation has torn most of the epithelium from its basement membrane. The cells seem to be atrophic. They are cuboidal instead of columnar, perhaps because of the distending and compressing effect of the fluid contents, and there may have been more than one layer. For the most part the cells are small and the cytoplasm takes more or less of the hematoxylin color, as though atrophic and alkaline.

The muscle is unstriated, occurs in loose fasciculi, is not uniform in thickness, and there is some adipose tissue beneath the tunica vaginalis.

Scattered about in the muscle tissue are numerous tiny canals

lined with cuboidal epithelium. Two sections of one of them are shown in the center of the little square (outlined in black) toward the corner of Figure 4, and are again shown highly magnified in Figure 5. These are regarded as vasa efferentia. Their position is not according to the normal anatomical plan, but it must be remembered that the structures are not only anomalous but also hypoplastic, so that deviations from the normal are to be expected.

Sections cut between the parallel lines at Figure 1 *h* were made in the expectation that they would pass through the seminal duct nearer the globus major. The duct was easily found and presented itself as shown in Figure 6. At this altitude it is lined with low columnar cells without cilia, is notably smaller in diameter than in Figure 4, and contains very little fluid. Again numerous tiny efferent ducts are distributed about, but the globus major is not found in the section.

A fragment of tissue was excised from the back of the mass of tissue Figure 1 *b*, referred to as muscular, at a position immediately behind the inner ends of the parallel lines at Figure 1 *h*, and a puzzling confusion of muscular bundles and epithelial tubules encountered. So difficult was it to account for the appearances that some friends, excellent pathologists, to whom the microscopic sections were shown and who at once agreed with all of the statements and conclusions thus far made and arrived at, felt it necessary to refer this mass to neoplastic growth. But this proved to be an error following the assumption that the larger end of the epididymis, that toward *e* (Fig. 1) was the globus major, or head of the organ, and the more tenuous part toward *g* (Fig. 1) the globus minor or tail that disappears in the muscular tissue to become the spermatic cord. As soon as the reverse of this conception is entertained and the globus major sought near where it should be, that is, in the neighborhood of the stalked hydatid *g* (Fig. 1) it becomes possible to understand that in the case of an imperfectly developed series of organs, one of which, the globus major of the epididymis, is formed by the confluence of many small tubules combining to form a single large one, considerable confusion of structure is not only possible but probable. With this thought in mind the original bewilderment fades away and the structure resolves itself into a mere modification of the duct system.

It is unfortunate that the gross material could not be dissected so that the course of the vas and spermatic cord could be traced. The described conditions of incision and fixation made this impossible.

The numerous sections of the testis studied revealed nothing corresponding to its mediastinum. Those from the cut surface *c* (Fig. 1) were uniformly tubular as shown in Figures 2 and 3, but those from the surface descending between the inner ends of the parallel lines at *h* (Fig. 1) show a tubular structure broken into smaller groups as the supposed neighborhood of the globus major is reached. The tunics seem to lose themselves by breaking into bands that confusedly enter the muscular tissue, and the tubular structure breaks up into groups of still convoluted tubules passing away from the testis proper. Apparently this is the region of the rete testis and vasa efferentia, though these structures are too inadequately developed to be definitely identified. One section, shown in Figure 8, appears much like the rete testis and may be its homologue. It may, therefore, be concluded that no globus major, as such, has been formed, though the essentials of its structure are scattered about.

It is also possible that no vas deferens exists except for the duct that occupies the length of the epididymis, the Wolffian ducts perhaps adopting the female course and development. If that be possible, it accounts for the seminal duct appearing to end blindly toward what is supposed to be the position of the globus minor. It also accounts for the absence of any definite spermatic cord, and the peculiarly irregular and distributed character of the muscular tissue of which it should be constituted.

In the shapeless muscular mass, the histology of which is shown in Figure 7, there is a cylindrical formation, *b* (Fig. 1), separated from the rest by a delicate fibrous investiture and at first promising as the homologue of the cord, but without the vas or any other epithelial canal or structure by which the homology can be established. It seems, therefore, improbable that any definite spermatic cord exists.

From these microscopic studies of the tissue little has been gained beyond the identification of a malformed and hypoplastic set of male sex organs, and the microscopic confirmation of the better formed parts as a testis with its epididymis.

Discussion and Conclusions

The discovery of a set of male sex organs in an otherwise perfect female can be interpreted only as an indication of true hermaphroditism, but as the subject of the deformity is living, only middle-aged and in good health, there is little probability that a postmortem examination can be made for many years. Under these circumstances it has seemed wise to record the interesting observation that accident has made possible.

The subject seems to be a true hermaphrodite. To consider her a pseudohermaphrodite would be to assume the existence of only one set of sexual organs, which would be those of the male, one-half of which were removed at the operation for hernia and subjected to examination: to consider her a true hermaphrodite would be to predicate the existence of female organs that have not been seen. But the perfect female habitus of the body, the absence of hirsuties, the soprano quality of the voice and above all the regularity of menstruation, leaving out of account the suspicion of the pregnancy and abortion of the early years of married life, are almost indubitable evidence of their existence and function.

No interstitial cells were discovered in any of the sections of the testis, though that does not preclude their presence, as only a very small proportion of the testis was examined and what the remainder might show is unknown. But, if present, they must have been inactive, for the body and its functions all point to the dominance of the endocrine influences of the glands of the opposed sex. It is scarcely conceivable that a male pseudohermaphrodite would have such perfectly formed female external genitalia and such perfectly developed secondary female characteristics as to appear, feel and behave as a female.

The operation at which the specimens were obtained was performed in the early part of 1932, but although the matter of endocrine alteration was carefully inquired into in the correspondence carried on with the surgeons, there is no evidence of any change in the patient, nor is it to be expected when it is considered that the removed male organs seem not to have shown the least potency during the years that they were contained in her body.

DESCRIPTION OF PLATES

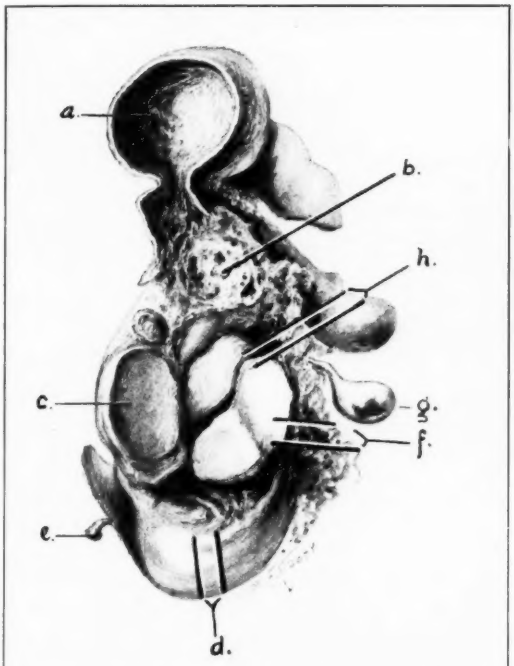
PLATE 85

FIG. 1. Semidiagrammatic representation of the removed tissue submitted for examination.

a = Supposed sac of the femoral hernia.
b = Mass of fibromuscular tissue supposed to be the homologue of the spermatic cord (see Fig. 7).
c = The testis (see Figs. 2 and 3).
d = The epididymis, the parallel lines showing the position of the portion excised for sectioning (see Figs. 4 and 5).
e = Cyst supposed to arise from the organ of Giraldes.
f = Parallel lines showing where another portion of the epididymis was excised for sectioning (see Fig. 6).
g = The stalked hydatid.
h = Parallel lines showing the position of the tissue, section of which proved to be the rete testis (see Fig. 8).

FIG. 2. Showing the perfectly regular tubular structure of the immature testis. See higher power view in Fig. 3.

FIG. 3. Higher power view of the section of the testis showing the tubules, with their well developed basement membranes and somewhat irregular spermatocytes. The immature development has not reached the stage at which primary and secondary spermatocytes, Sertoli cells, and spermatozoa are found.



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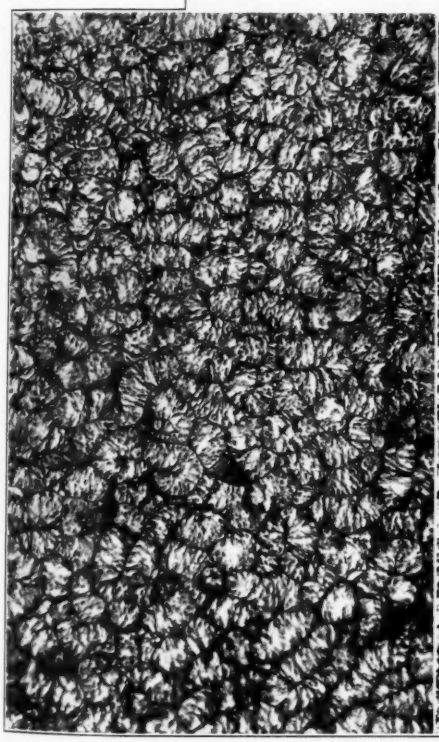

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McFarland

Malformation Assumed to be True Hermaphroditism

Plate 86

Fig. 4. Section through the body of the epididymis showing the vas, muscular coats and vessels of the pampiniform plexus, with a section through one of the vasa efferentia in the square. See Fig. 5, a higher magnification of the tissue in the square.

Fig. 5. Higher magnification of the marked square shown in Fig. 4, showing sections of one of the vasa efferentia of the epididymis.

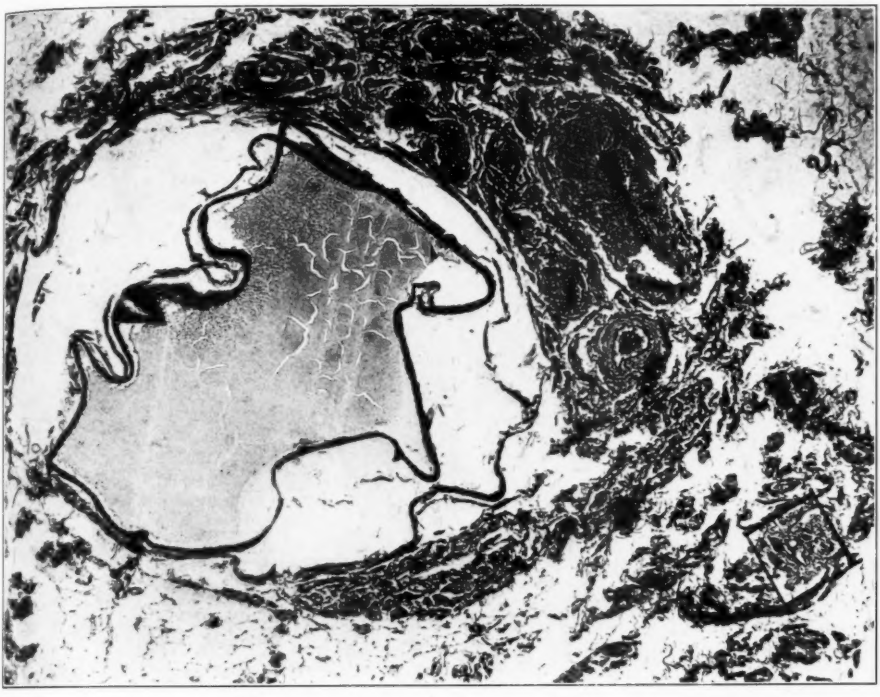

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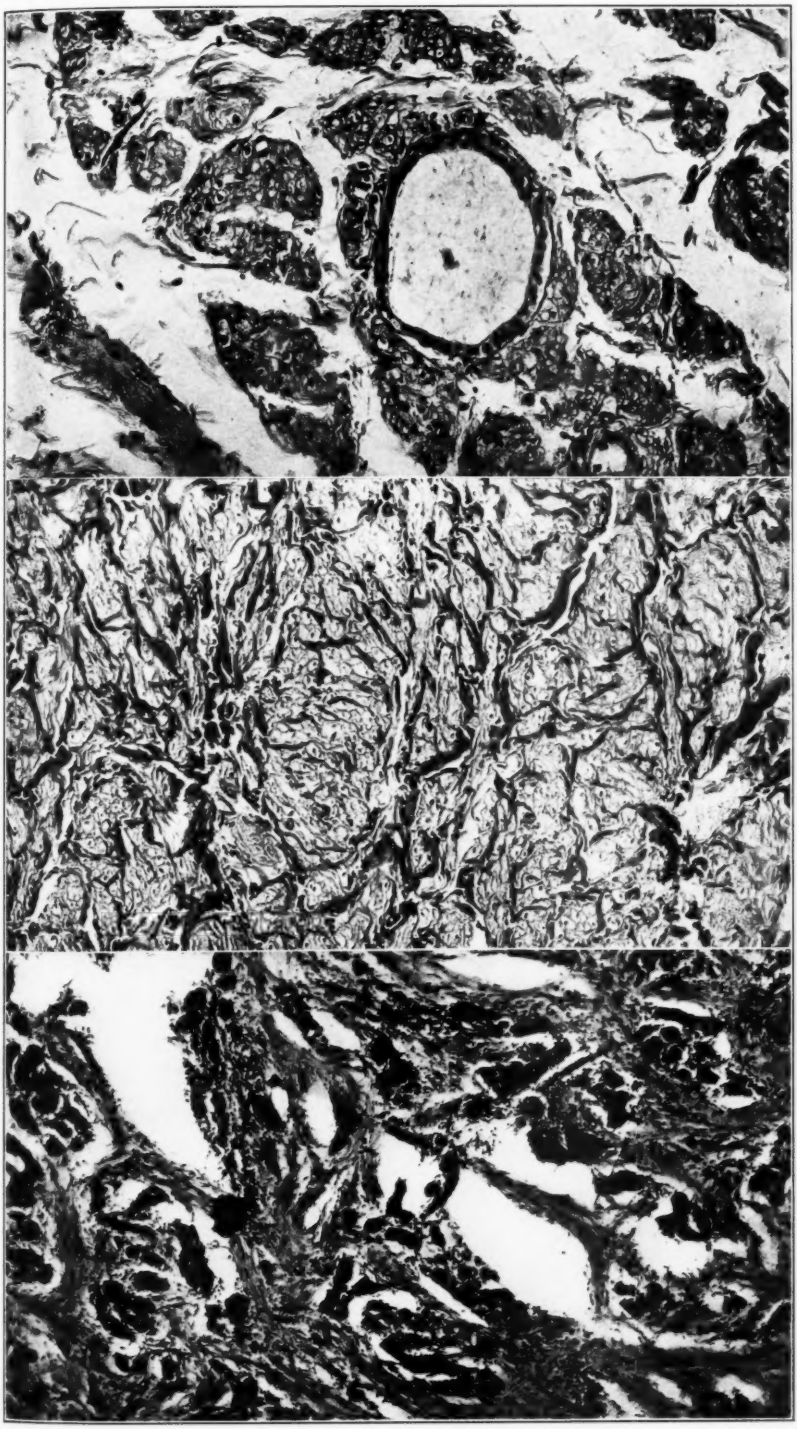
PLATE 87

FIG. 6. Section through the upper (distal) part of the vas deferens.

FIG. 7. Fibromuscular tissue supposed to be the homologue of the spermatic cord.

FIG. 8. Section through the area supposed to correspond to the rete testis, near the normal origin of the globus major of the epididymis. Bad fixation has caused retraction of the loosely attached epithelium from the spaces it normally lined, and has almost completely closed the lumens.



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McFarland Malformation Assumed to be True Hermaphroditism

PHOSPHORUS AND ALCOHOLIC CIRRHOSIS *

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(From the Pathological Laboratory of the Boston City Hospital, Boston, Mass.)

INTRODUCTION

The object of this paper is to present a certain amount of evidence in favor of the view that so-called alcoholic cirrhosis may be due to chronic poisoning with phosphorus. The idea was first put on record [1] in the Shattuck Lecture delivered before the Massachusetts Medical Society, June 8, 1932.

The work reported here represents the culmination of thirty-six years' search for the cause of this type of cirrhosis. At an autopsy which I made May 13, 1897 on an adult male there was found a 2350 gm. cirrhotic liver in which microscopically over half of the cells contained in their cytoplasm masses of hyaline acidophilic material (Fig. 1). The lesion in the liver cells was so striking and peculiar that a definite cause for it seemed inevitable. The problem which in time suggested itself was to find that cause.

The search started mildly enough but for the past eighteen years has been an obsession. After ruling out, like other investigators, ethyl alcohol as the cause, every possible toxic substance (over 30 in all) which could be imagined as contaminating liquors was investigated experimentally. The list includes methyl and amyl alcohols, oil of juniper, turpentine, creosote, salicylic and pyroligneous acids, furfurol, sulphites, lead, copper, tin, aluminum, antimony and arsenic.

I worked at various times on the problem with three of my former assistants, Drs. McJunkin, Parker and Nye, but of recent years alone. Most attention was paid to lead and arsenic because when given in large amounts they produced acute lesions that seemed promising. This was particularly true of lead, which causes in a very few days the formation of a hyaline basophilic reticulum in liver cells, as reported by McJunkin [2] in 1915. Recently I found that a similar lesion was formed at the same time in many of the renal cells. But with small doses given over a long period of time no

* Received for publication June 5, 1933.

comparable pathological changes could be produced, although the attempt to do so was made repeatedly under varying conditions.

During the past five years increasing attention has been paid to phosphorus as the possible cause of so-called "alcoholic cirrhosis," in spite of the fact that no obvious connection between it and liquors was evident. It was tried at first for two reasons: its well known injurious effect on the cytoplasm rather than on the nuclei of liver cells, and the observation that the cytoplasm of the liver cells in a case of phosphorus poisoning contained eosinophilic granules. Experimental work soon showed two strong points in its favor. It readily produces cirrhosis, and when administered in small enough doses over a long enough time gives rise to peculiar degenerative changes in the cytoplasm of the liver cells. The evidence obtained at first was purely morphological but it was promising. In order to appreciate its significance it is necessary to describe first the characteristic and diagnostic lesion of alcoholic cirrhosis.

Alcoholic Cirrhosis

The commonest type of cirrhosis of the liver is that known as alcoholic cirrhosis, because it is so frequently associated with overindulgence in alcoholic beverages. In our series of cases at the Boston City Hospital covering thirty-six years, and all verified by microscopic examination, it forms about 50 per cent, as shown in the accompanying table.

Table I

Cases of Cirrhosis at the Boston City Hospital (1897–1932 Inclusive)

Total number of autopsies during the past 36 years ... 10,016
Total number of cases of well marked cirrhosis of the liver ..... 590 = 5.89 per cent

They are classified as follows:

	No. of Cases	Per Cent
1. Alcoholic cirrhosis	293	49.66
2. Pigment cirrhosis	50	8.47
3. Healed acute yellow atrophy	48	8.13
4. Syphilitic cirrhosis	28	4.74
5. Colon bacillus cirrhosis	27	4.57
6. Obstructive cirrhosis	27	4.57
7. Obstructive and colon bacillus cirrhosis	6	1.08
8. Cancer cirrhosis	1	0.16
9. Unclassified	110	18.66

The diagnosis of this variety of cirrhosis is based on the presence of a fine to coarse acidophilic reticulum in the cytoplasm of the

liver cells. With prolonged staining, especially after fixation in alcohol, the reticulum can be stained fairly deeply basophilically both by methylene blue and by alum hematoxylin. The lesion is sometimes found before any evidence of cirrhosis is present and occasionally complicates pigment cirrhosis. In livers in which the cirrhotic process is very active the hyaline reticulum may be present, as in the first case studied, in over half of the cells, but when the process is very chronic only a few affected cells may be found and these are usually old cells at the periphery of the islands of regeneration.

The lesion in the liver cells is, as already stated, both characteristic and diagnostic. Old decolorized bile occurring in liver cells as the result of bile stasis may become acidophilic, but occurs as separate, round, hyaline globules of various sizes (Fig. 4). This type of lesion is sometimes found associated with true hyalin in cases of alcoholic cirrhosis in which focal bile stasis has occurred. The reticulum produced by very acute lead poisoning is basophilic and of very rare occurrence. But one example of it has occurred in my personal experience.

The degenerative changes occurring in the cytoplasm of the liver cells are found throughout the lobule but are usually most marked, at least in early cases, around the central vein. The cirrhotic process on the other hand starts in the portal regions and extends irregularly into the lobule.

Fatty infiltration of the liver cells is a frequent complication of alcoholic cirrhosis and, especially in the earlier stages of the process, often causes marked increase in the size of the organ.

The lesion of alcoholic cirrhosis begins as very fine acidophilic granules in the cytoplasm of the liver cells. They enlarge, coalesce and form a hyaline reticulum that often surrounds the nucleus (Figs. 5 and 6). The reticulum varies according to age and rapidity of formation (Figs. 1, 2 and 3) from fine to coarse, and eventually loses its marked acidophilic property so that it stains lightly or not at all. If the cell dies, the reticulum is attacked by polymorphonuclear leukocytes or monocytes and gradually dissolved. As I have previously stated, I have always regarded the reticulum as the fingerprint of the agent that causes this type of cirrhosis. It is the only clue to its identity that we possess.

Study has shown that the acidophilic granules and reticulum have

certain physical and chemical properties. They stain deeply with eosin (phloxine) by the eosin-methylene blue method after fixation in Zenker's fluid. They stain even more intensely by the following simple method after fixation in alcohol (80 to 95 per cent).

Phloxine Stain for Alcoholic Hyalin

1. Stain paraffin or celloidin sections rather lightly in alum hematoxylin.
2. Wash in water.
3. Stain in a 0.5 per cent solution of phloxine in 20 per cent alcohol for 1 or more hours. Place in incubator at 37° C if necessary.
4. Wash in water.
5. Transfer to a very dilute solution of ammonia water (1 drop of a strong (28 per cent) solution to 100 cc. of water) for 1 to 2 minutes.
6. Wash in water.
7. Decolorize in 95 per cent alcohol, followed by absolute alcohol and xylol for paraffin sections or by oleum origani cretici for celloidin. Mount in Canada balsam.

Nuclei blue, hyaline reticulum and granules intense pink to bright red.

The granules and reticulum also stain deeply by the Macallum [3] and Fiske-Subbarow [4] methods for phosphates. Both methods depend on the formation of a phosphorus-molybdenum compound that is rendered prominent through its reduction by certain reagents to the blue oxide of molybdenum.

Macallum Test for Phosphates, as Used in these Experiments

1. Alcohol fixation (80–95 per cent): celloidin embedding.
2. Place section in following mixture in incubator at 37°C for 24–48 hours. Make two solutions. Add *A* to *B* slowly with constant agitation to prevent formation of insoluble precipitate.

A. Molybdic acid (H_2MoO_4) ..... 1 gm.
(Do not use anhydride form, MoO_3)
Ammonia water, S.G. 0.88 ..... 4 cc.
Water ..... 4 cc.
Stir until salt is dissolved.

B. Nitric acid, S.G. 1.2 (33 per cent) ..... 15 cc.
Water ..... 15 cc.

3. Wash in water 1–2 minutes.
4. Place in a 2 per cent aqueous solution of phenylhydrazine hydrochloride for 2–10 minutes.
5. Wash in water, 95 per cent alcohol, origanum oil, balsam.

Fiske-Subbarow Test for Phosphates

1. Alcohol fixation: celloidin embedding.
2. Two solutions are necessary.
   A. 2.5 per cent ammonium molybdate made up in 5 N sulphuric acid.
   B. 0.25 per cent aqueous solution of aminonaphtholsulphonic acid.
3. Place sections in a mixture of the two solutions, combined in the proportions of 10 cc. of *A* to 4 cc. of *B*, in the incubator at 37° C for 24–48 hours.
4. Wash in water, 95 per cent alcohol, origanum oil, balsam.

Certain clinical aspects of alcoholic cirrhosis are of importance. It is very rare before the age of 25 years and is most common in the fourth, fifth and sixth decades. This late occurrence of the process, although indulgence in liquor often begins much earlier, argues that the condition is of slow development, requiring ordinarily some five to fifteen years or more for its production. On the other hand, it sometimes occurs in children and it has been noted as occasionally affecting more than one child in a family. I have studied sections from the livers of a girl 12 years of age and of her brother aged 17 years, both of whom died at the Massachusetts General Hospital from typical chronic alcoholic cirrhosis. No history of indulgence in alcohol was obtained and it is unlikely to have occurred at that early age.

One gains the impression from the study of the disease that it is much more common among the poorer people who drink the cheaper, less carefully prepared and less aged brands of liquor.

Although investigators are generally agreed that alcoholic cirrhosis is not due to ethyl alcohol (all experimental work is against it) it is well recognized that there is a very close relation between

alcoholic beverages and the disease. This fact suggests strongly that the injurious agent is a contaminating substance contained at times in liquors.

Phosphorus Cirrhosis

Much attention has been paid to the marked fatty changes and necrosis produced in the liver by acute poisoning with phosphorus, and several observers have stated that the cytoplasm of the liver cells is more affected than the nuclei. Chronic poisoning on the other hand has attracted practically no notice and Wegner [5] (1872) seems to be the only experimenter on record who has produced cirrhosis in this way.

In my experimental work the greatest difficulty has been to determine the dosage and time required to produce the effects desired. So far 85 rabbits and guinea pigs have been used. The results obtained can be summarized fairly briefly.

Cirrhosis of the liver can easily be produced by phosphorus in three months or more (Figs 11 and 12) and is often complicated by ascites and jaundice. The daily dose required is two-thirds to one milligram of phosphorus to each kilogram of body weight. A 0.2 per cent solution of yellow phosphorus dissolved in oil of sweet almonds is convenient to use because 1 cc. equals 2 mg. of phosphorus. By means of a small syringe the exact amount required for each animal is readily measured and then injected slowly into the mouth of the animal held in the vertical position. The solution causes no discomfort or irritation. For the more chronic type of lesion a 0.1 per cent solution will be found preferable.

Phosphorus produces apparently two effects on the liver. It injures two different types of cells, the fibroblasts of the stroma, especially in the region of the portal vessels (Fig. 11), and the liver cells throughout the lobule. Destruction of fibroblasts is followed by regeneration, as shown by the occurrence of occasional mitoses, and by fibrosis, which is periportal in the beginning but soon extends irregularly into the lobule (Fig. 13). The fibrosis of the liver is easily produced, leads to marked increase in consistence and gives the liver a finely granular surface, usually most noticeable on its under surface.

If the dose of phosphorus is large (1 mg. to 1 Kg. of body weight), the liver cells are destroyed in great numbers and regeneration is

very active. Mitotic figures are numerous. If the daily dose is reduced to about 0.33 mg. to 1 Kg. of body weight, degenerative changes slowly take place. Eosinophilic granules appear in the cytoplasm, enlarge and fuse together to form a hyaline reticulum that stains acidophilically. The shortest time required to produce the degenerative changes characteristic of alcoholic cirrhosis was four and a third months (Figs. 8, 9 and 10). Even in this liver mitotic figures were present (Fig. 10). A smaller dose would seem to be preferable (0.25 mg. to 1 Kg. of body weight), but six months to a year or more might be required to produce well marked lesions.

Satisfactory degenerative changes are much more difficult to produce than the fibrosis because a smaller dose and much longer time are required.

Attempts to produce similar degenerative changes in liver cells by the use of certain poisonous compounds of phosphorus, such as ethyl and diethyl phosphine and calcium phosphide, gave only negative results. For their preparation and the opportunity to test them I am greatly indebted to the kindly interest of Dr. Harry M. Weber, chemist.

The lesion characteristic of alcoholic cirrhosis would seem to be due to the element yellow phosphorus itself and not to any of its compounds.

Discussion

As already stated, the diagnosis of alcoholic cirrhosis is based on a characteristic degenerative change occurring in the cytoplasm of the liver cells. It consists of a hyaline reticulum formed from granules that enlarge and coalesce. The granules and the reticulum are strongly acidophilic. They also give the reaction for phosphates by the Macallum and Fiske-Subbarow microchemical staining methods.

It is possible by the daily administration of very minute doses of yellow phosphorus dissolved in oil of sweet almonds to produce a definite cirrhosis in rabbits and guinea pigs in three months or more. If the dosage is made smaller and continued over a longer time, so that the liver cells degenerate very slowly instead of undergoing necrosis, a series of retrograde changes similar to those found in alcoholic cirrhosis and reacting microchemically in the same way

slowly takes place. Acidophilic granules appear in the cytoplasm, enlarge and fuse to form a hyaline reticulum.

These experimental results afford morphological and microchemical evidence that alcoholic cirrhosis may be due to phosphorus. The natural question is — how can the patient take phosphorus into his system? One possible way is obvious. Phosphorus is administered therapeutically chiefly under three conditions, (1) in rickets, (2) to promote callus formation, and (3) in certain nervous disorders. Its use in rickets may possibly explain the rare occurrence of alcoholic cirrhosis in children, but certainly throws no light on the frequent occurrence of the disease in adults.

All available evidence points strongly to the conclusion that this most common type of cirrhosis is connected in some way with excessive indulgence in strong liquors. Is it possible that they sometimes contain phosphorus?

Naturally the greatest objection to the suggestion of phosphorus as the cause of alcoholic cirrhosis lies in the difficulty of explaining how it can get into alcoholic beverages — its probable source. Evidently it is not deliberately put there. If it is ever present, it must be as an accidental contaminant. Up to the present time only one possible source has suggested itself and that is iron.

Phosphorus is present in certain iron ores, sometimes in considerable amount, and thus gets into pig and cast iron and also into iron and steel made from them. It is said to exist there as iron phosphide and also as an alloy of iron. Iron utensils and cans made from tin plate might be conceived therefore as yielding phosphorus if eroded by acids, which are commonly present in liquors. If set free, the phosphorus would probably be preserved, at least for some time, in water and even better in alcohol just as it is in oils. This idea was adopted as a working hypothesis and various substances including a limited number of liquors tested.

Tests for Phosphorus: Phosphorus is characterized chiefly by two properties, phosphorescence and its reaction with silver salts. Unfortunately neither is diagnostic. Phosphorescence was discarded early in this experimental work because it does not afford a sufficiently delicate test and is prevented by the presence of alcohol.

The silver test employed in toxicology is performed by suspending a strip of paper moistened with nitrate of silver in the neck of a flask containing the suspected fluid. The apparatus is warmed gently at a

temperature of 30 to 40°C (according to Autenrieth 40–50°C) for several minutes. The flask is closed by a perforated cork to permit the slow escape of vapor, which is carried over the surface of the paper. Phosphorus turns the paper light to dark brown (silver phosphide). Hydrogen sulphide, however, produces much the same effect (silver sulphide). Therefore a second strip of paper moistened with acetate of lead is also suspended in the flask. If only the silver paper is browned, phosphorus is present; if both papers are browned, hydrogen sulphide is present and phosphorus may be. No method for differentiating the effects of these two substances on the silvered paper from each other is given. An attempt to find one resulted in the following.

Photographic printing paper was found to be more convenient and sensitive than the paper moistened with silver nitrate. It is turned light to dark brown in a few minutes to 24 hours by phosphorus, depending on its strength, and light to dark brown by hydrogen sulphide. Placed in a solution of permanganate of potassium acidified with sulphuric acid (equal parts of a 0.25 per cent solution of the first and of a 2 per cent solution of the acid), the paper is decolorized in 1 to 5 minutes, depending on the intensity of the color. The phosphorus compound is the more readily changed. After washing in water the paper, now colored brownish by the potassium permanganate, is placed in the Fiske reagent which promptly decolorizes it and, if phosphorus is present, gradually turns it blue in the course of 5 to 10 minutes. Wash in water, place in the ordinary hypo (sodium thiosulphate) solution for 2 to 5 minutes to remove any soluble silver salts present, wash thoroughly in water and dry. If hydrogen sulphide was present, the paper may be colored a very light brown. After the first step, which is best performed in the dark, the test may be carried out in ordinary light.

The method, which was arrived at only after much experimentation, was tried out first with water saturated with phosphorus (1:33,000) using a 24 hour exposure of the paper to the vapor at a temperature of 25°C. Diluted 1:10 (1:330,000) a positive reaction could still be obtained and alcohol did not interfere with the test.

Tested in the same way, 10 per cent hydrochloric acid gave a negative result, as did also chloride of iron, iron sulphide and iron phosphide, all of which were dissolved in 10 per cent hydrochloric acid. On the other hand, a strongly positive reaction was obtained

with cast iron chips, C. P. iron (alcoholized, Eimer and Amend), and tin plate (cut into small bits so as to expose as much iron surface as possible), all dissolved in 10 per cent hydrochloric acid.

These reactions would seem to indicate that phosphorus is commonly alloyed with iron and is set free as the element when iron is dissolved in an acid, or at least in dilute hydrochloric acid. It evidently is not derived from the iron phosphide.

Up to the present time the test has been applied to 25 samples of hard liquor with entirely negative results.

Certain technical points in connection with the demonstration of phosphorus may be of use. I have employed Gevaert's Nova Brom photographic printing paper almost exclusively in these experiments.

If the paper is developed with a metol-hydroquinone solution after exposure to phosphorus very minute quantities may be demonstrated. The same is true of hydrogen sulphide. The method, however, is much more delicate than that employing silver nitrate paper or than the Fiske reaction. Possibly it may be developed into a diagnostic test later.

Developed by the metol-hydroquinone solution, the paper acted on by phosphorus turns gray to intense black, while that exposed to hydrogen sulphide retains a brownish tinge.

The acid permanganate and Fiske solutions should be combined immediately before use and not employed for a second lot of paper.

Summary and Conclusions

Very chronic poisoning with minute daily doses of yellow phosphorus produces, in five months or more, cirrhosis of the liver in rabbits and guinea pigs.

The liver cells undergo degenerative changes similar to those found in alcoholic cirrhosis. They contain granules and hyaline networks (reticula) that are acidophilic and give the reaction for phosphates by the Macallum and Fiske-Subbarow tests.

By a special method it is possible to demonstrate free phosphorus when cast iron and tin plate are dissolved in dilute hydrochloric acid.

Phosphorus may get into alcohol and liquors through acid erosion of iron and tin plate vessels and containers and thus contaminate them and be the cause of alcoholic cirrhosis.

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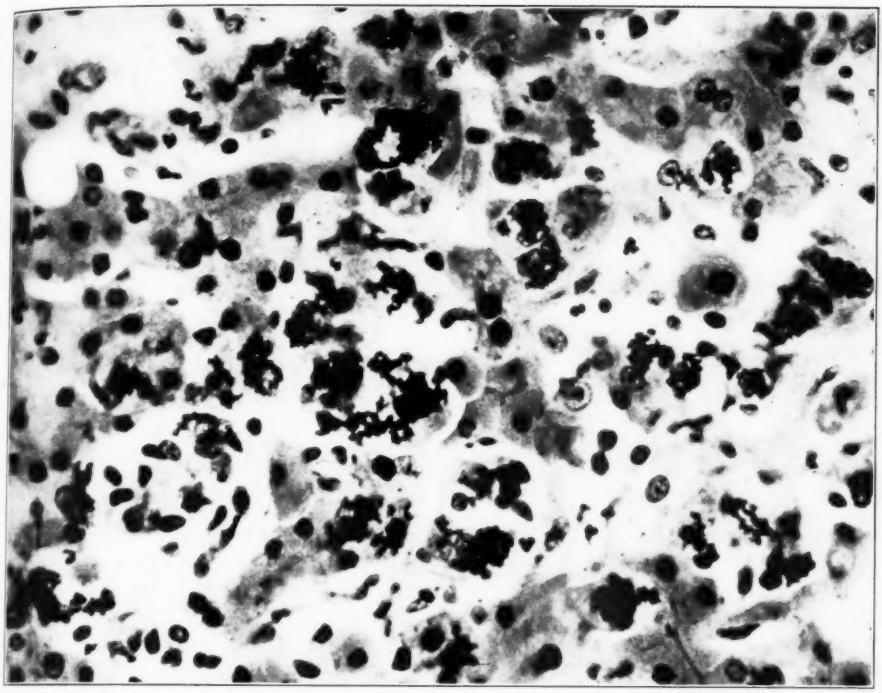
DESCRIPTION OF PLATES

PLATE 88

FIG. 1. A-97-172. Alcoholic cirrhosis; first case observed and studied. Many of the cells contain coarse masses of acidophilic material (hyaline network or reticulum) in their cytoplasm. × 500.

FIG. 2. U-30-33. Alcoholic cirrhosis — a chronic case. Nearly every liver cell in an island of regeneration is filled with fairly coarse hyaline material. × 100.



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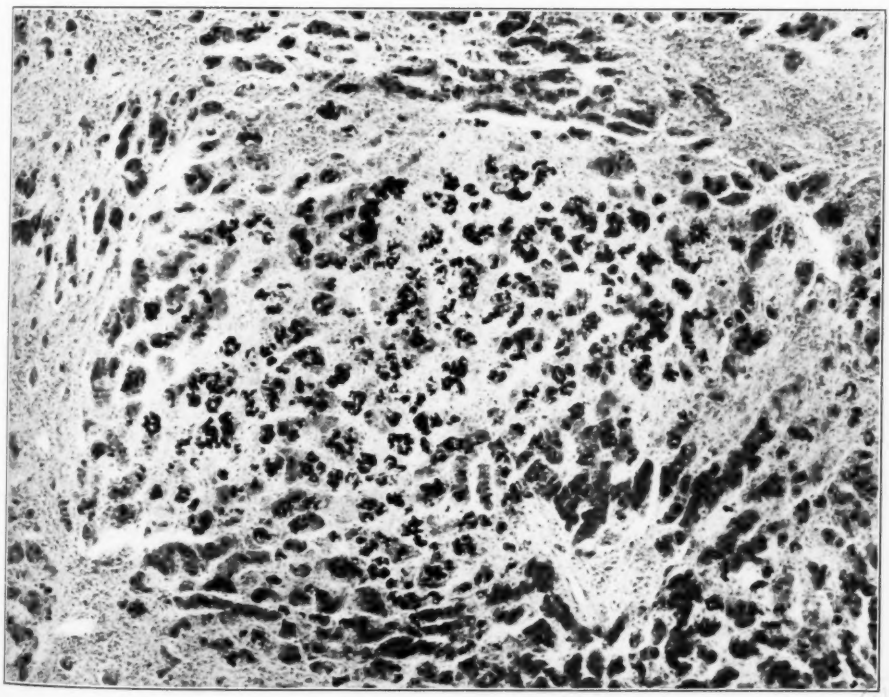


2

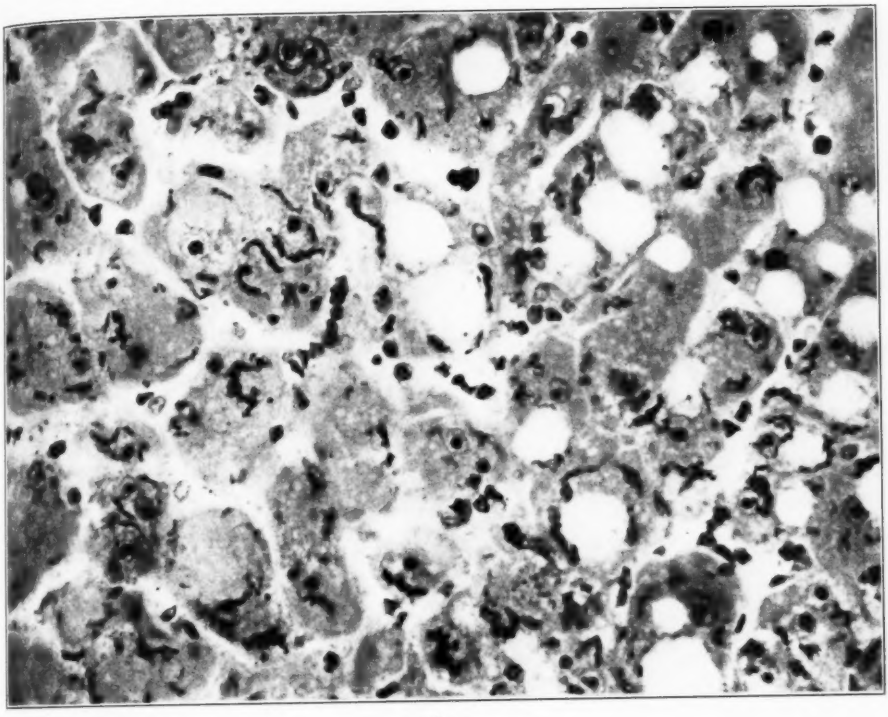
Mallory

Phosphorus and Alcoholic Cirrhosis

PLATE 89

FIG. 3. A-01-21. Alcoholic hyalin present in nearly every liver cell in the form of granules and delicate reticula. No cirrhosis evident. The lesion is probably of very slow formation. × 500.

FIG. 4. U-27-10. Bile stasis. The inspissated bile in the cytoplasm of the liver cells is in the form of variously sized droplets and has been decolorized and become acidophilic so that it stains deeply with eosin. × 500.



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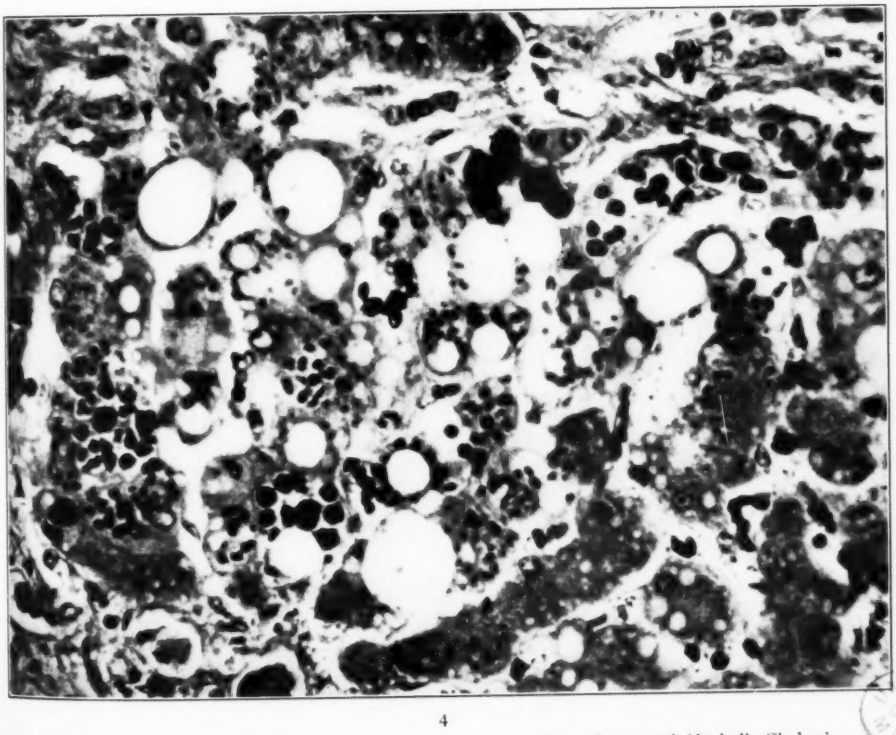

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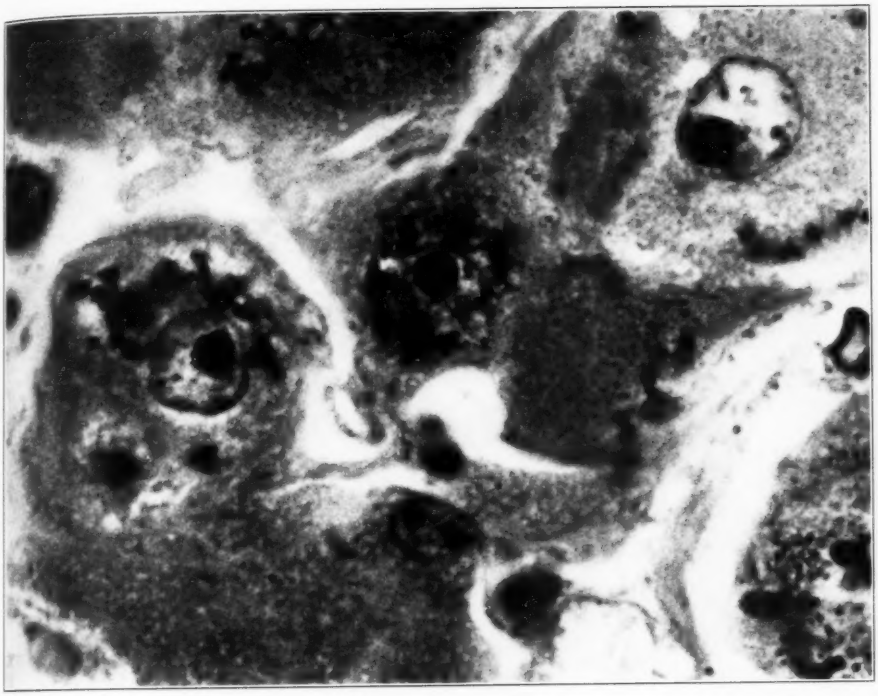
Mallory

Phosphorus and Alcoholic Cirrhosis

PLATE 90

FIG. 5. A–01–21. Alcoholic hyalin in the process of development from eosinophilic granules which enlarge and coalesce to form a network in the cytoplasm, sometimes surrounding the nuclei. × 2000.

FIG. 6. A–01–21. Another example of hyalin forming from eosinophilic granules in the cytoplasm of liver cells. × 2000.



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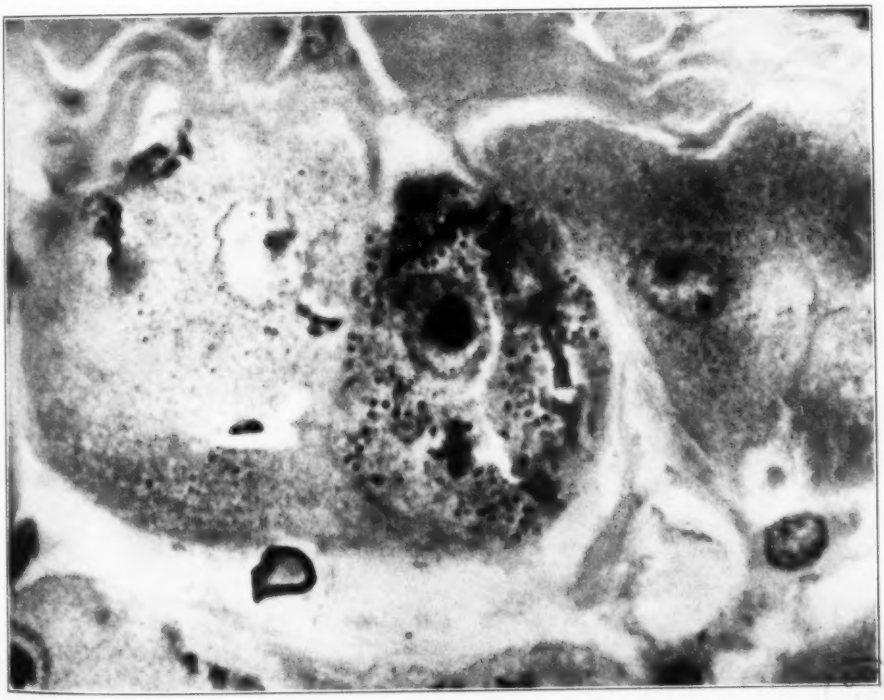


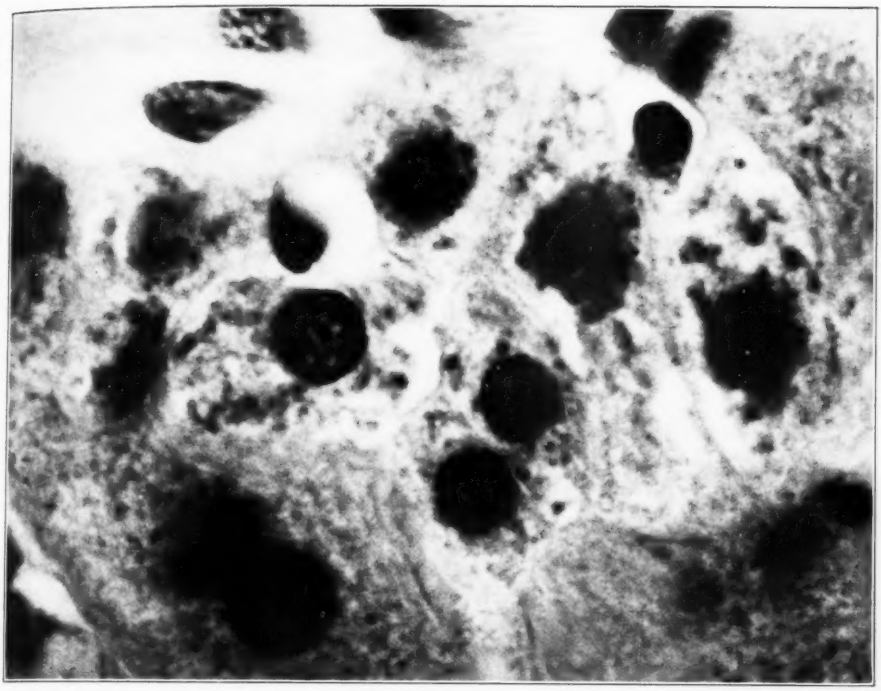
6

Mallory Phosphorus and Alcoholic Cirrhosis

PLATE 91

FIG. 7. Z801. Phosphorus cirrhosis, $4\frac{1}{3}$ months duration. Average daily dose of phosphorus 0.25 mg. to 1 Kg. of body weight. Early stage showing eosinophilic granules beginning to appear, enlarge and fuse to form hyaline material. × 2000.

FIG. 8. Z780. Phosphorus cirrhosis, $4\frac{1}{3}$ months duration. Dose of phosphorus given daily 0.33 mg. to 1 Kg. of body weight. Every cell contains hyaline material in the process of forming a network more or less surrounding the nuclei. × 2000



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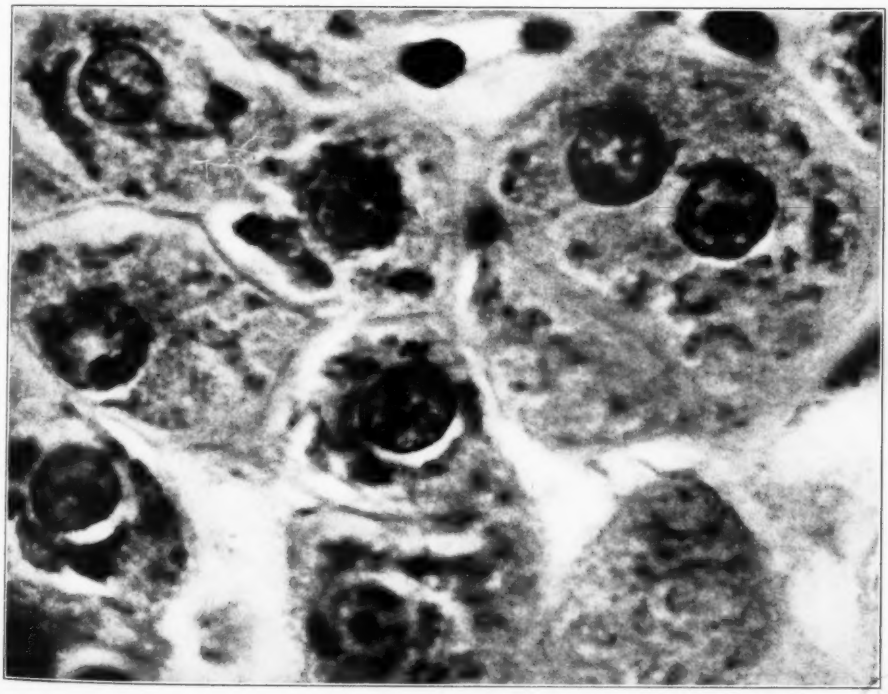

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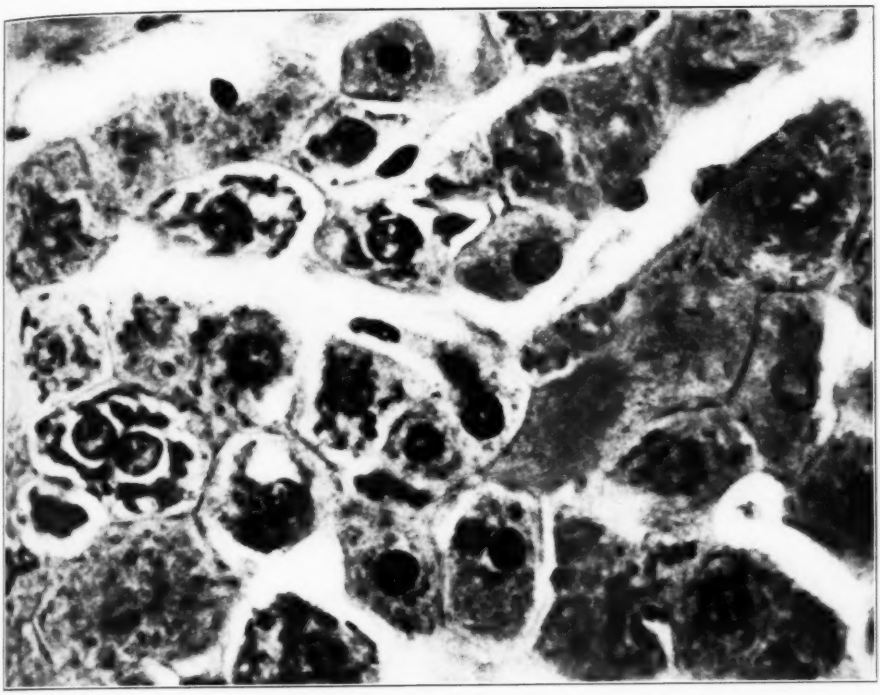
Mallory

Phosphorus and Alcoholic Cirrhosis

PLATE 92

FIG. 9. Z780. Phosphorus cirrhosis. Nearly every cell contains granules and hyaline reticula that stain intensely with phloxine. × 1000.

FIG. 10. Z780. Another field from the same liver as in Fig. 9. Two mitoses are present, one in the upper center and the second in the upper left corner. × 1000.



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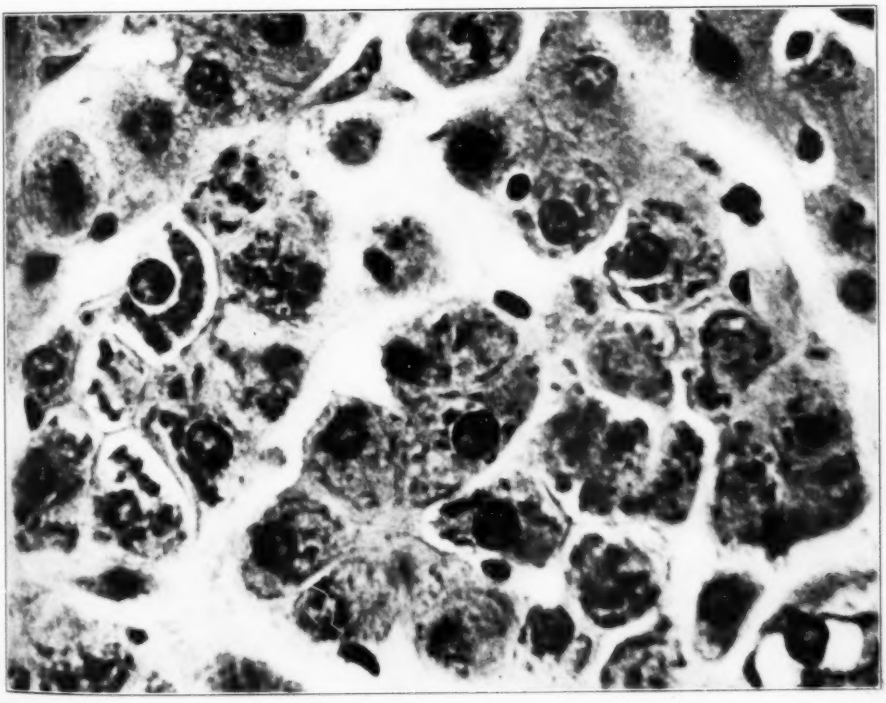

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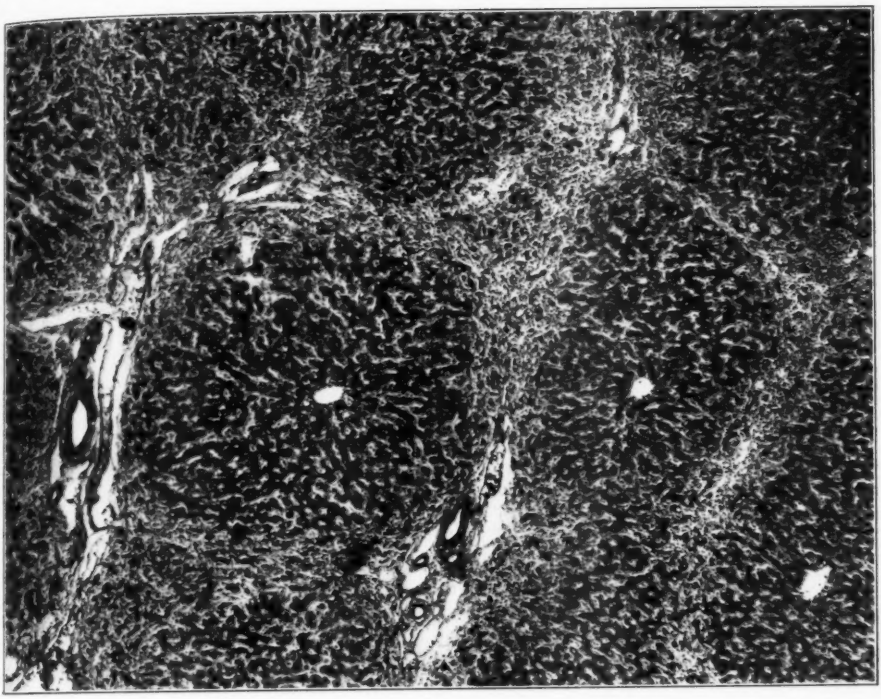
PLATE 93

FIG. 11. Z737. Phosphorus cirrhosis produced in 8 weeks. Daily dose 1 mg. to 1 Kg. of body weight. Cirrhosis distinctly periportal in origin and distribution. × 50.

FIG. 12. Z738. Phosphorus cirrhosis produced in 11 weeks. Similar in character to Z737 (Fig. 11).



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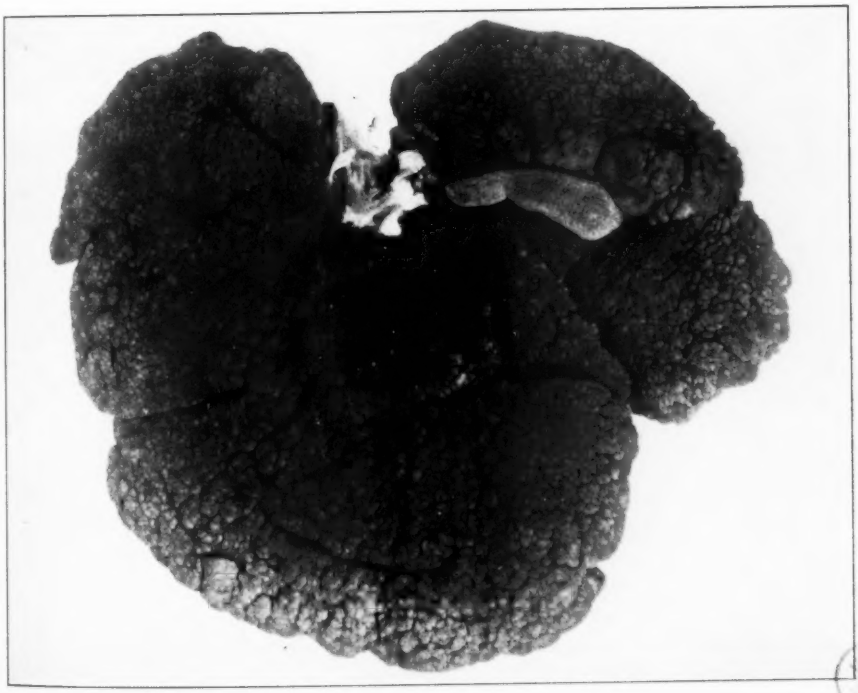

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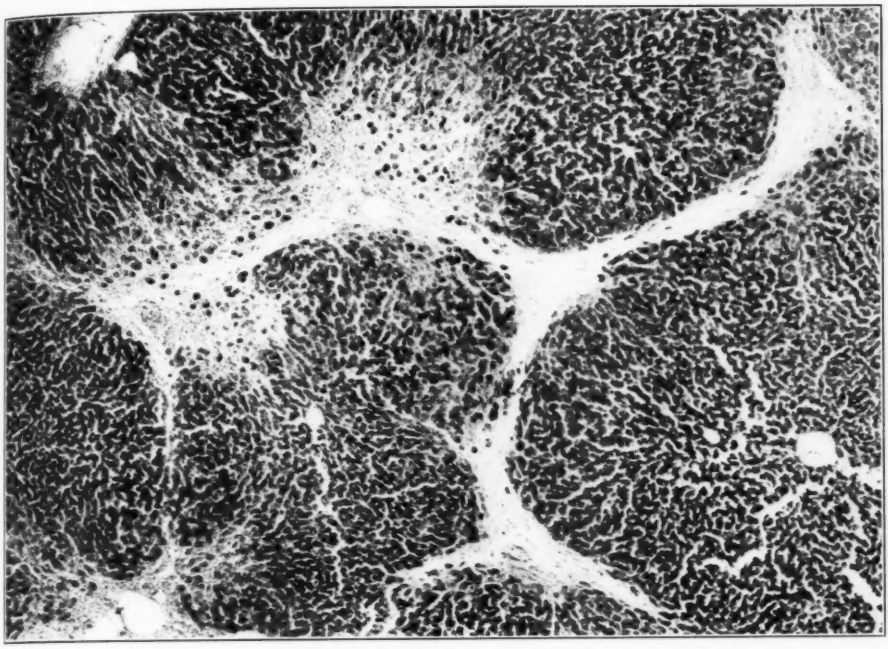
Mallory Phosphorus and Alcoholic Cirrhosis

PLATE 94

FIG. 13. Z780. Phosphorus cirrhosis. Low power view to show the degree of sclerosis present. It is periportal in origin but is extending irregularly into the lobules. Details from this liver are pictured in Figs. 8, 9 and 10. × 50.

FIG. 14. Z780. Phosphorus cirrhosis. Gross appearance of under surface of liver



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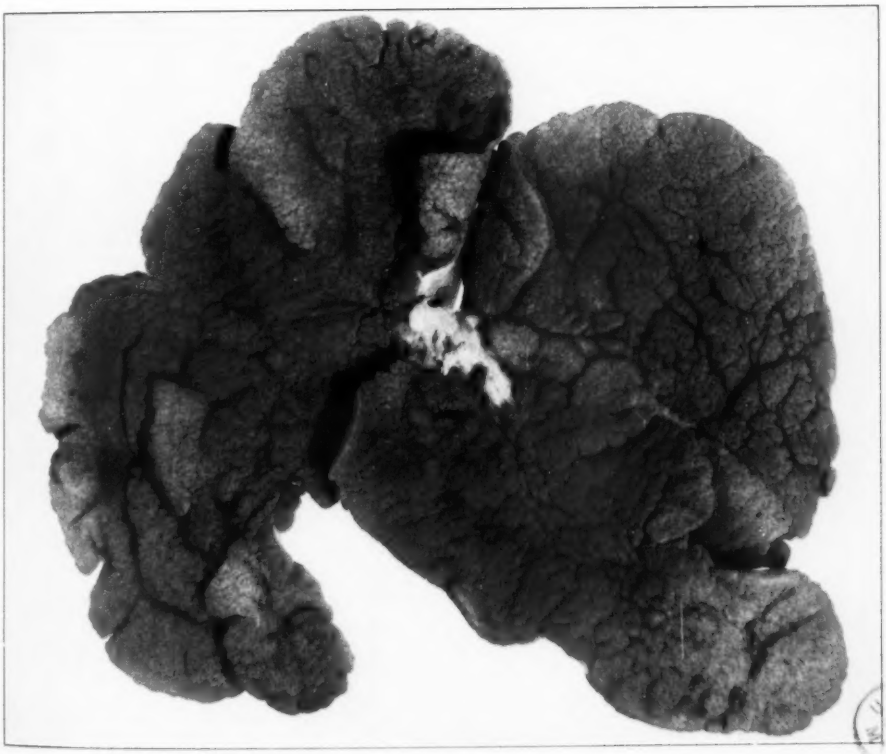

14

THE PERMEABILITY OF THE KIDNEY TO BACTERIA *

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In spite of considerable experimental investigation there is still doubt as to whether or not the normal uninjured kidney can discharge bacteria coursing in the circulation. Although it is true that the majority of authors contend that such an "excretion" occurs only after definite lesions have been produced in the kidney, one constantly encounters even in the recent literature such statements as "the ability of the normal kidney to excrete any organisms that may, from time to time, reach the blood-stream." The present study is a reexamination of the problem with particular emphasis on the exact localization of the bacteria in the kidney after their injection into the circulation.

The first published statement on the subject was that of Cohnheim,[1] who said that "not only the dissolved constituents of the blood, *but some undissolved ones* (including bacteria) *are capable of excretion by the kidneys*, and then make their appearance in the urine secreted by healthy kidneys when and because they are contained in the blood." This opinion was soon challenged by Wyssokowitsch (1886),[2] who found that various spores and bacteria which did not affect the kidneys were absent from the urine for periods up to 24 hours after injection into the blood stream of dogs and rabbits. Even *Staphylococcus aureus* and *B. anthracis* did not appear until several hours after injection, when the kidneys were already the seat of recognizable lesions. He used different methods of obtaining the urine. During life the animals were catheterized; after death, in males, the urine was expressed from the bladder through the urethra, while in females the abdomen was opened, a hole burned through the bladder wall and a pipette inserted.

In 1887 Schweizer,[3] using a "green-producing bacillus" for injection and a ureteral fistula for obtaining the urine, found the organisms in the urine frequently within a few minutes after their intro-

* Received for publication April 5, 1933.

duction into the blood stream. He suggested that bacteria settling in the kidney might damage a few cells in such a way as to permit their escape into the urine, although such lesions were not demonstrable in stained sections. Sherrington (1893),[4] in a careful study of the presence of bacteria in several of the body secretions, showed that they occurred only after an interval sufficient to result in damage to the secreting membranes.

The article by Biedl and Kraus,[5] which appeared in 1896, has been an important factor in fortifying the opinion that the kidney can readily excrete bacteria. These workers injected *Staphylococcus aureus*, *B. coli* and *B. anthracis* into dogs and rabbits and, on culturing the urine, often found the organisms 5 to 15 minutes after the administration. Their usual method of recovering the urine was by cannulae inserted into the ureters. Their results were far from consistent and they were careful to point out that there was nothing regular in the appearance of the bacteria in the urine, as one might expect in a physiological process. Their experiments were confirmed by von Klecki [6] and Opitz,[7] although the latter raised several important theoretical considerations against the view that the normal kidney could excrete bacteria.

Dyke (1923) [8] studied the changes in the kidney taking place at various intervals after the intravenous injection of *Staphylococcus aureus* and, although he could demonstrate a reaction of the tissues as early as 1 hour after the injection, the cocci did not appear in the urine till the 5th hour; by this time there were very definite inflammatory changes in the kidney. In a thorough and convincing series of experiments Helmholz and his associates [9-15] showed that various organisms, virulent and avirulent, did not pass into the urine within the time usually considered adequate for excretion, but that inflammatory changes and mechanical interference with the urinary system could break down the kidney filter for bacteria.

The above is only a representative selection of writers who have performed experimental investigations of this problem, but it serves to indicate the contradictory results and opinions. Comparatively few attempts have been made to reconcile the different results. Jahn [16] found that the urine of a number of species of mammals at times had a bactericidal effect on some of the common pathogenic organisms. He suggested, therefore, that those urines giving negative results within a few minutes after the intravenous

injection of bacteria may have had a destructive effect on the small number of organisms that had passed through. Lepper [17] thought that the manipulation necessary to secure the ureteral catheters which were used by Biedl and Kraus and by Opitz may have led to a temporary increase in pressure in the pelvis of the kidney. This would cause compression of the renal veins, congestion of the kidney and consequently slight hematuria. The most significant work in this connection was that of Helmholz and Field.[11] They compared the results of their first series of experiments, in which the urine was obtained through a seared portion of the bladder wall, with a second group in which the urine was withdrawn through a catheter and was thus liable to contamination by blood, due to the trauma of the catheterization. In the latter the urine contained organisms in the first 2 or 3 hours after injection in nearly 50 per cent of cases. Also, the irregularity in time of appearance and number of bacteria in the second series is strongly suggestive of the findings of Biedl and Kraus and of von Klecki.

A careful study of the literature inevitably leads to the conclusion that the crux of the matter, in so far as the experimental investigations are concerned, is the method of obtaining the urine. It is readily apparent that any procedure in which there is any possibility of contaminating the urine with blood is liable to lead to false conclusions. This is particularly true in the first few minutes after injection, when the blood is teeming with organisms. And, of course, this is just the period when the recovery of the bacteria in the urine makes the "excretion" seem so similar to that of a dye like methylene blue, which appears in the urine within 2 or 3 minutes after introduction into the circulation. Some workers have attempted to rule out contamination by blood by examining the urine chemically, but this is open to the objection that the usual tests are not sufficiently delicate to detect a minute amount of blood which might nevertheless contain a considerable number of bacteria.

Just how much the various operative procedures, anesthesia, presence of toxic material in the broth of cultures, number of organisms injected and other factors affect the kidneys is difficult to judge, but it seems safe to assume that the less interference with the animal in the interval between the injection of the bacteria and the recovery of the urine the more likely that the results will be reliable. On this account, after a thorough analysis of the many variations in tech-

nique previously employed, the writer decided the method that promised most in the elimination of possible sources of error was that in which one animal was used for each experiment, and the urine obtained by cauterizing a portion of the bladder wall and withdrawing the contents with a syringe.

The examination of ordinary histological sections to determine the exact location of a few isolated microörganisms in the tissues is an exceedingly laborious task, particularly when it is realized that a cross-section of the rabbit's kidney, measuring 2 to 3 square cm., may contain only one or two bacteria. An even more important consideration is that the results of such an examination may be unreliable, as will be pointed out later. To overcome these objections, the method of first incubating the tissue, in order to permit the growth of the individual bacteria into easily discernible colonies, was adopted.

Experimental Study

Male rabbits between the ages of 6 and 12 months were used in all the experiments. Some days before the injection of the organisms the animals were catheterized and the urine examined for evidence of spontaneous kidney infection.

Three organisms were used: *Staphylococcus aureus*, *Bacillus coli* and *Bacillus prodigiosus*. The staphylococcus was obtained from a human case of osteomyelitis of a tarsal bone and the colon bacillus from a case of pyelitis in a child. To eliminate the possible toxic effects of bacterial products present in broth cultures, saline suspensions were used throughout. Each organism was grown in Hartley's broth plus dextrose for 18 to 24 hours and then suspended in physiological saline so as to form a distinctly turbid mixture. The McFarland nephelometer was used to obtain emulsions of approximately equal turbidity for experiments on different days. In order to get rid of large clumps of organisms and so avoid the possibility of embolism, the suspension was centrifuged at low speed for a few minutes before use. No attempt was made to determine the number of bacteria — it was deemed sufficient that a very large number of organisms be introduced into the blood stream.

Injection was made into the marginal vein of the ear, and after the desired interval had elapsed the animal was killed by a blow on the neck. To guard against postmortem evacuation of the bladder (a

mishap that resulted in the sacrifice of several animals before it was detected), the urethra was gently compressed between the pubis and the soft tissues overlying it by an assistant. Using aseptic technique the abdomen was opened and the bladder exposed. A relatively avascular portion of the wall was cauterized with a special instrument and the urine withdrawn through this area with a needle and syringe.

The urine thus obtained was divided into two samples. The first and larger was centrifuged at high speed for 30 minutes and the supernatant fluid drawn off and tested for albumin. A loop of the sediment was streaked on an agar plate, while the balance of the sediment was used to make an agar pour plate. To facilitate the identification of colonies, blood agar or plain agar was used in the experiments in which staphylococcus had been injected, Endo agar for *B. coli* and plain agar for *B. prodigiosus*. The sediment was also examined microscopically. The second sample of urine was used to test for possible bactericidal effect on the organism. For this purpose the urine was seeded with a loop of the suspension used for the injection and incubated at 37.5° C for a variable period, usually 24 hours, but never less than the time interval between the injection and the death of the animal. Transplants to agar plates were made immediately after the seeding and after the period of incubation. All the cultures were read after 3 days.

At the same time as the urine was obtained from the bladder the left renal vessels and ureter were tied off, the kidney removed to a sterile Petri dish and incubated. The kidneys in the staphylococcus and colon bacillus experiments were kept at 37.5° C for 18 to 20 hours, while in the experiments with *B. prodigiosus* the kidneys were incubated at 30° C for 24 hours. The right kidney was immediately placed in fixative (10 per cent formalin in the staphylococcus experiments and Zenker's solution in the others). After the period of incubation the left kidney was also fixed. Paraffin sections were cut and stained by a modification of the Gram-Weigert method.

Results of Experiments

The findings in the bacteriological examination of the urine are collected in the accompanying tables. It will be noted that staphylococci first appeared in the urine in the 12 hour interval. There was

a striking relation between the number of organisms in the urine and the time interval after injection. In Rabbit 4 there were about 50 colonies in both plates. In Rabbit 273 the number was much increased but the individual colonies were easily seen, while in Rabbits 461 and 462 the colonies were so numerous and so small that in the pour plates they were not visible to the naked eye.

TABLE I

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

I. Staphylococcus Aureus

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
	cc.			
454	1	10 min.	None	+
453	1	35 "	"	+
452	1	1 hour	"	+
451	1	2 hours	"	+
463	1	3 "	"	+
33	1	5 "	"	+
32	1	8 "	"	+
4	1	12 "	Few	+
273	1	18 "	Many	+
461	1	24 "	Innumerable	+
462	1	48 "	"	+

Other workers who do not support the theory of the excretion of organisms by the uninjured kidney have found staphylococci in the urine in as short a time as 5 hours after injection. Probably this difference in the time of the first appearance of the cocci is related to the degree of virulence of the strain and so to the rapidity with which lesions are developed in the kidney. A rabbit injected with 1 cc. of the suspension survived for 13 days, indicating that this strain was not very pathogenic as compared with others that kill an animal in a few hours.

In the series of animals injected with the colon bacillus the urine was negative even up to 48 hours after the injection. (The one organism in the urine of Rabbit 841 (Table II) was probably a contamination; it is difficult to conceive of a lesion in the kidney permitting a single bacterium to escape into the urine). Some other investigators have found the bacilli in the urine as early as the 7 hour

interval, so that here, too, the organism used was not very virulent. But the kidneys were damaged to some extent, even though not sufficiently to cause bacilluria: this is shown by the fact that from 12 hours onward the urine contained such abnormal constituents as granular casts and a fair number of epithelial cells. That the colon bacillus varies considerably in its ability to affect the kidney is a point noted by several writers (Lepper,[17] Helmholz and Beeler[18]).

TABLE II

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

II. Bacillus Coli

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
	cc.			
9	2	10 min.	None	+
28	2	30 "	"	+
24	2	1 hour	"	+
436	2	2 hours	"	+*
15	2	4½ "	"	+
493	2	8 "	"	+
5	2	12 "	"	+
380	2	19 "	"	+
841	2	24 "	1 colony	+
6	2	48 "	None	+*

* The second transplant to agar showed a smaller number of colonies than the first, from which it may be concluded that these urines had a slight inhibitory effect on the colon bacillus.

B. prodigiosus did not reach the urine in any of the experiments, which was to be expected in view of the failure of a pathogen like *B. coli* to pass the renal membrane.

The right hand columns of the tables show that in a few cases the urine proved to be a poor culture medium for the injected organism. Thus the bactericidal effect of some urines on bacteria must always be kept in mind in experimental work of this nature, but that it plays as large a part as Jahn claimed does not appear to be the case. In a few instances the organisms failed to withstand exposure to the urine, but in the majority of cases they not only survived but multiplied rapidly.

The above results are not in accordance with any theory which holds that bacteria are "excreted" by the kidneys. For true excre-

tion to take place the bacteria should appear in the urine within a few minutes after introduction into the circulation and persist until they are practically all removed from the blood.

TABLE III

Bacteriological Examination of Urine at Varying Intervals after the Intravenous Injection of Bacteria in Rabbits

III. Bacillus Prodigiosus

Rabbit No.	Amount of suspension injected	Interval between injection and death of animal	Organisms in urine	Survival of organisms in inoculated urine
	cc.			
98	2	15 min.	None	+
99	2	30 "	"	+
95	2	1 hour	"	+
38	5	3 hours	"	+
96	5	5 "	"	+
34	5	8 "	"	+
2	5	12 "	"	+
381	5	18 "	"	Not done
1	5	24 "	"	—

EXAMINATION OF SECTIONS

Comparatively little attention has been devoted in this work to the development of lesions in the kidney after the injection of bacteria into the blood stream, since this has been well described on previous occasions. My interest has been chiefly in the determination of the exact location of the organisms in the renal substance. For this purpose the careful study and interpretation of the sections of incubated kidneys have proved to be most interesting and instructive.

A few typical fields in these sections are reproduced in the photomicrographs. In order to understand them properly it will be necessary to take into consideration a few simple facts. Bacteria growing within a confined space will naturally form colonies that conform in shape more or less to that of the space. For instance, if they are growing within a hollow sphere they will tend to form an approximately globular colony; if they are in a tube they will form a cast of the tube, and so forth. The peculiarities of the particular organism will also play some part. Thus a motile organism growing in a tube

will extend for some distance along the tube, while a staphylococcus will remain more definitely localized. Other factors also enter, such as the suitability of the nutriment, compactness of the tissues, and so on. For the sake of comparison, a section of kidney with the blood vessels injected with gelatin is shown in Figure 1.

Figure 2 illustrates a typical colony of *B. coli* growing within the capillary loops of a glomerulus. The well rounded outline of the bacterial clumps is quite characteristic. The delimiting walls of the colony, though not apparent in the photograph, are readily seen under the microscope. The shape of the colony alone, however, is sufficient evidence that the bacilli are lying within the capillaries of the tuft. It is only reasonable to conclude, then, that the bacillus from which this colony originated lay *within* a capillary at the time of death. In contrast, if the organism had penetrated to the capsular space, the resulting colony would appear on section in the shape of a ring or crescent. *Such a colony was never seen in any of the experiments with B. coli or B. prodigiosus.*

A colony of colon bacilli growing between tubules is seen in the next photograph. Theoretically, there are three possible sites for such a colony: (1) an intertubular capillary, (2) a lymphatic vessel, and (3) the tissue spaces. The first is almost certainly the probable one, but there is no way of proving it, since the containing wall, being so thin, cannot be positively identified. One thing is certain, though, and that is that the organisms are *not* within the lumen of one of the tubules, which are, of course, very thick-walled.

There is no doubt when a colony such as is shown in Figure 4 is encountered. Here the bacilli are seen growing among the red cells in a blood vessel.

The difficulty of determining in just what type of vessel the organisms lie is exemplified by a colony in the medulla, Figure 5. It is quite plain that the bacteria are growing within some sort of thin-walled tube, for the latter is situated alongside some tubules that are being compressed. One might argue that in this part of the kidney such a colony might be within the thin-walled portion of a loop of Henle. If that were the case, one would expect to find colonies further on in the course of the nephron, in a distal convoluted tubule or collecting tubule; such colonies were not seen, however.

The appearance of the colonies of staphylococci is much the same as of the colonies of colon bacilli. Because of the non-motility of the

cocci the colonies tend to be more or less spherical, whereas the motile bacilli often progress for some distance along the capillaries. This is well shown in Figure 6, where only about 3 capillary loops are filled, compared to the 8 to 10 occupied by the bacilli in Figure 2. The shape of the clumps of cocci makes it certain that the growth took place *within* the blood vessels and not in the space between the two layers of Bowman's capsule. Figure 7 shows a colony of cocci as it appears in the medulla. If it had grown in one of the large collecting tubules, such as surround it, there would have been no difficulty in recognizing the fact.

The colonies of *B. prodigiosus* are very similar to those of the colon bacillus.

The photomicrographs so far described were all taken from sections of kidneys removed within the first hour after the injection of the bacteria. In examining a large number of stained sections one occasionally comes across a colony, the exact position of which it is difficult to analyze, due to its large size, distortion of neighboring structures, and so on. But it may be reiterated here that in none of the *B. coli* or *B. prodigiosus* experiments were bacteria seen growing *within* the lumen of what could be definitely identified as part of the tubular system. In order to visualize such colonies it is necessary to examine kidneys from the experiments with staphylococci, where definite lesions have been established. Figure 8, from Rabbit 462, which was killed 48 hours after the injection of *Staphylococcus aureus*, shows clearly two colonies lying within the lumina of tubules. During the incubation of the kidneys the tissues undergo considerable autolysis; this renders somewhat indistinct the cytological detail, so that at times it is not easy to tell to what part of the tubular system a particular portion belongs. There can be no doubt, however, that these colonies lie within portions of the convoluted tubules. It is not possible to say how much of each colony was present at the time of death and how much was due to growth in the incubator. Apart from other considerations, such a figure is exceedingly useful in that it furnishes a guide to the appearance of colonies of organisms within the tubules.

An interesting study in contrasts is presented in Figure 9. This, too, is from Rabbit 462. It shows an intense inflammatory reaction, consisting of a central zone of necrotic tissue and a ring of polymorphonuclear leukocytes — a developing abscess. Five malpighian

corpuscles are seen in the central area, *A–E*. In one, *A*, the cocci have burst through the walls of the tufts and are growing all through the cavity. *F*, a tubule containing organisms, probably originates in *B*. Particularly noteworthy is the corpuscle *E*, containing clumps of organisms with smooth, rounded outlines; this glomerulus should be compared with those shown in Figure 2 and Figure 6. It is apparent that in spite of the fact that this glomerulus must have been severely damaged, situated as it was in the middle of the abscess, the walls have not yet given way, although there is a suspicion of a rupture at *G*. Such a picture emphasizes the strength of the endothelial membrane, and suggests the thought that if the capillaries (or the visceral layer of Bowman's capsule) were able to hold out against the cocci for 48 hours during life and 20 hours in the incubator, it is not likely that the normal capillary endothelium and the renal epithelium covering it would permit the passage into the capsular space of one or two cocci within a few minutes after their injection into the blood stream.

This method of locating bacteria in the kidney would appear at first sight to be an infallible one, but it must be confessed that there is one, at least, possible source of error. As a control measure it is necessary to know whether or not the organisms would multiply postmortem and so show up by this method of investigation if they should manage to get into the lumen of the *normal* renal corpuscle or tubule. One cannot adduce such a picture as Figure 8 as a solution, because in that case the kidney had been severely damaged by the infection and many of the abscesses had ruptured into the tubules, permitting the presence of such abnormal constituents as albumin and blood, and making available a better food supply for the organisms than that of the fluid present in the normal tubules. Nor can one assume that because the bacteria grew well in the bladder urine in most cases (see tables) they would necessarily thrive in the tubules, for the fluid in the first part of the nephron is of quite a different character from the urine that issues from the papillary ducts into the pelvis of the kidney. The glomerular filtrate undergoes great changes in salt content, volume and pH as it passes through the tubules, changes that affect materially its suitability as a culture medium.

In an attempt to learn whether or not the organisms would grow in the normal tubules, saline suspensions of *Staphylococcus aureus*

and *B. prodigiosus* were injected up the ureters of a pair of kidneys freshly extirpated from the body of a rabbit. This manoeuver failed, either because the bacteria did not grow in the tubules, or because they never got that far. The latter is the more likely explanation — it is a well known fact that it is very difficult to get substances into the interior of the kidney by injection through the ureter.

The Shifting of Small Particles in Histological Sections

Several investigators, more particularly those who claim that the normal kidney excretes bacteria, have described finding the organisms actually lying in the capsular space and in the lumens of the tubules on microscopic examination. For instance, Schweizer,[3] after pointing out how few organisms are to be found in sections, says: "I found the microörganisms to be most abundant by far in the glomeruli and in the space between them and Bowman's capsule. Then I found them fairly frequently in the blood vessels and in the lumen of the first part of the convoluted tubules. More rarely I saw them in the perivascular connective tissue. Only once could I with certainty demonstrate a bacillus between two epithelial cells of a convoluted tubule. Despite zealous searching I have never been able to find a coccus or a bacillus in the cells themselves."

Again, von Klecki[6] says: "The bacteria could be demonstrated microscopically by thionin staining. For the most part only single organisms were encountered — in the glomerular loops, in the capsular space of the glomeruli, in the lumen of the first portion of the convoluted tubules, and mostly between the vessels accompanying the straight tubules; only once was a bacillus found between two epithelial cells of a convoluted tubule. Thus the glomeruli constitute the chief pathway for the excretion of bacteria through the kidneys: The bacteria penetrate the vessel wall of the glomerular loops, reach the capsular space, then the convoluted tubules and are washed out with the urinary flow."

Vincenzi,[19] too, observed a few individual bacilli in the space bounded by Bowman's capsule as well as in the glomerulus. "After this discovery," he states, "it was clear to me that the excretion of the organisms injected into the blood-stream had taken place through the glomerulus." Rolly[20] did not see any bacteria in the sections, they were so few in number.

These observations, of course, fit in very nicely with a theory which holds that the normal kidney can pass bacteria, and it would seem impossible to reconcile such findings with the views of those who believe that the organisms do not get through to the urine in the first few hours after injection, and that they appear in the urine only after the renal tissues have been injured. We believe that there is a possible explanation, which depends on the fact that in the preparation of stained sections small portions of tissue are often displaced slightly from their original site. For example, on the edge of practically every paraffin section one may see small fragments of tissue, a single red cell or leukocyte, apparently floating in space — obviously detached at some stage in the preparation of the section. There is no reason why bacteria in the interior of sections should not be similarly affected, and that this does occur is shown in Figure 10, which shows the same glomerulus as in Figure 6 under a higher magnification. Note the cocci lying within the capsular space; any observer, seeing one or two such organisms would say, and with perfect justification, that they were lying between the parietal and visceral layers of Bowman's capsule and definitely outside the vascular system. But the shape of the colony makes it practically a certainty that the bacteria had grown within the capillaries and that before fixation they were all confined within the capillary loops. At some stage in the cutting and staining of the section, however, a few of the cocci were dislodged from the main mass. This movement, small though it may be, is of tremendous importance in a problem of this kind, for it means the difference between an organism lying within a capillary and within the lumen of Bowman's capsule. The same phenomenon is shown in Figure 11, which shows a colony of cocci in the medulla. Again, the shape of the colony indicates that the organisms grew out in a tubular structure, but the displacement of a few of the cocci is plainly apparent at the point indicated by the arrow.

This explanation would be valueless if each section contained great numbers of organisms, but such is not the case. Everyone who has attempted the search for the individual bacteria has commented on the surprisingly small number to be found, even though enormous numbers were injected. Indeed, this rapid disappearance of the organisms from the blood stream is most graphically shown in a series of incubated kidneys. Ten minutes after the injection a

cross-section of the kidney is studded with colonies, up to 150 in number. This number rapidly decreases, depending on the number and type of organism injected, until at 8 hours a similar cross-section shows only one or two colonies. It is now an accepted fact that this primary sterilization of the blood stream is performed mainly by the cells of the so-called reticulo-endothelial system.

It was noted that the shifting of a few bacteria from the main mass was nearly always in the same direction in each section. At just which step in the preparation of the sections this displacement takes place it is not possible to say definitely at present. A few experiments carried out with one paraffin block of tissue cut in different directions seemed to indicate that during sectioning the microtome knife would push a few of the bacteria away from their original resting place for a short distance. Another possibility is that minute portions of the tissue might float around in the Canada balsam before the latter has hardened. At any rate, these sections show that small particles of matter like bacteria can and do become displaced to such an extent as to lead to errors in deciding their exact situation.

Discussion

It will readily be apparent from what has been said above that there is more to this problem than the mere injection of bacteria into the blood and the subsequent determination of their presence in the urine. The bacteriological method of examining the urine is almost too subtle, particularly when liquid culture media are used, for, obviously, it does not distinguish between organisms that have passed through the kidneys and any that may have reached the urine because of faulty technique. For this reason such procedures as those employed by Biedl and Kraus and others, who inserted cannulae into the ureters in order to obtain the urine, are always open to question.

Not only do the results obtained in this work show that the normal kidney is not permeable to bacteria, but they also tend to indicate that the damage to the renal membranes must be of considerable degree before the organisms can pass through. More evidence in favor of this view is found in the results of a few experiments, not recorded in the tables, in which bacteria were injected into animals whose urine contained considerable albumin. The time of appear-

ance of the bacteria in the urine did not differ from those cases in which the urine was albumin-free. It would seem that organisms can penetrate into the urine through lesions that they themselves have caused, but do not necessarily pass through more readily if the kidney is already damaged by some other agent. Other workers have noticed the same phenomenon. Wyssokowicz [21] first induced acute nephrosis in animals by injecting such irritants as ammonium bichromate, cantharides, and so on, and, after a few days, injected the bacteria. In spite of the damage to the renal parenchyma, as shown by the presence of albumin and casts in the urine, the organisms were not excreted by the kidneys. Similarly, Helmholz and Bowers [15] found that the presence of experimentally produced acute staphylococcic lesions in the kidneys did not predispose to the rapid passage of colon bacilli into the urine.

Although this investigation is intended to be mainly an experimental study, it may not be amiss to refer to one or two points that are of clinical interest. Probably the most important practical application of this problem is in renal tuberculosis. It has been known for some time that there are certain patients with tuberculosis in parts of the body other than the kidney whose urine contains tubercle bacilli, as shown by guinea pig inoculation. This occurs in the complete absence of symptoms referable to the urinary tract — "silent" bacilluria. Moreover, when these cases come to autopsy, not a few of them show absolutely nothing on ordinary gross and microscopic examination of the kidneys. The knowledge of these facts has had considerable to do with "confirming," on the clinical side, the theory that the normal kidneys can pass bacteria.

Closer examination of all the circumstances throws considerable doubt on this claim. Much of the misunderstanding has arisen because tuberculosis of the kidney is usually considered in terms of gross lesions, principally of two varieties: the large caseating type, and that caused by miliary dissemination. But a point often overlooked is that miliary tuberculosis may be of all degrees, from an extremely mild, clinically unrecognizable form to one in which the dissemination is heavy and widespread, causing profound systemic reaction and ending inevitably in death. In other words, the number of tubercle bacilli that gain access to the blood stream may vary from a few to countless myriads. Hence it is easy to conceive of a light shower of bacilli resulting in a few very small lesions in the

kidneys, which discharge bacilli into the urine for a time and then heal, all without symptoms and without evidence at postmortem. This conception is supported by a study made a few years ago in this laboratory by Shipley, who examined the kidneys in some cases of tubercle bacilluria. The patients had complained of no urinary symptoms, the only signs of renal disease being the presence of the bacilli and a few pus cells in the urine. At autopsy no lesions were evident either in the gross or in the usual microscopic examination of a few blocks of tissue. It was only after serial section of considerable portions of the kidneys was performed that definite microscopic tubercles were found, which were quite sufficient to account for the bacilluria.

Summary and Conclusions

1. *Staphylococcus aureus* injected into the circulation of normal rabbits did not appear in the urine until between 8 and 12 hours later.

2. *B. coli* was absent from the urine even up to 48 hours after injection.

3. *B. prodigiosus* failed to reach the urine up to 24 hours after injection.

4. The examination of ordinary paraffin sections to determine the exact site of a few individual bacteria is not entirely reliable.

5. The method of incubating the kidney before fixation and staining not only facilitated greatly the finding of the bacteria, but permitted a more dependable analysis of the location of the organisms from which the colonies had developed.

6. By means of such examination it was seen that the bacteria never reached the capsular space about the glomeruli or the lumens of the tubules in the first few hours after injection, hence were not excreted. The probability is that they did not get beyond the vascular system until they had damaged the walls of the latter.

It is a pleasure to acknowledge the many kindnesses of Prof. Oskar Klotz, in whose department this work was carried out. The author is also greatly indebted to Prof. W. L. Holman, Dr. E. J. Clifford, Mr. Walter Cowan and many others in the Banting Institute for their suggestions and assistance.

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DESCRIPTION OF PLATES

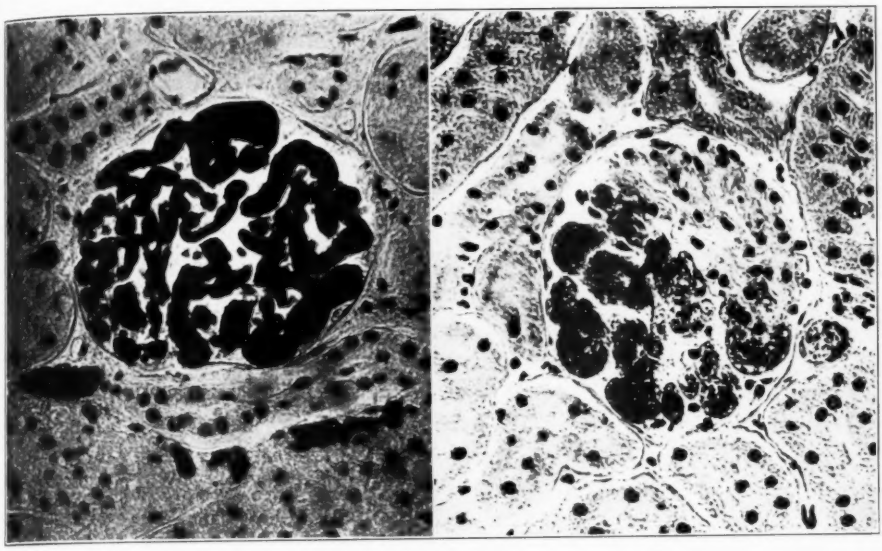
PLATE 95

FIG. 1. A section of a cat's kidney with the blood vessels injected. × 410 (approximately).

FIG. 2. A colony of *B. coli* in a glomerulus. × 410.

FIG. 3. A colony of *B. coli* between convoluted tubules. × 410.

FIG. 4. A colony of colon bacilli within a blood vessel. × 410.



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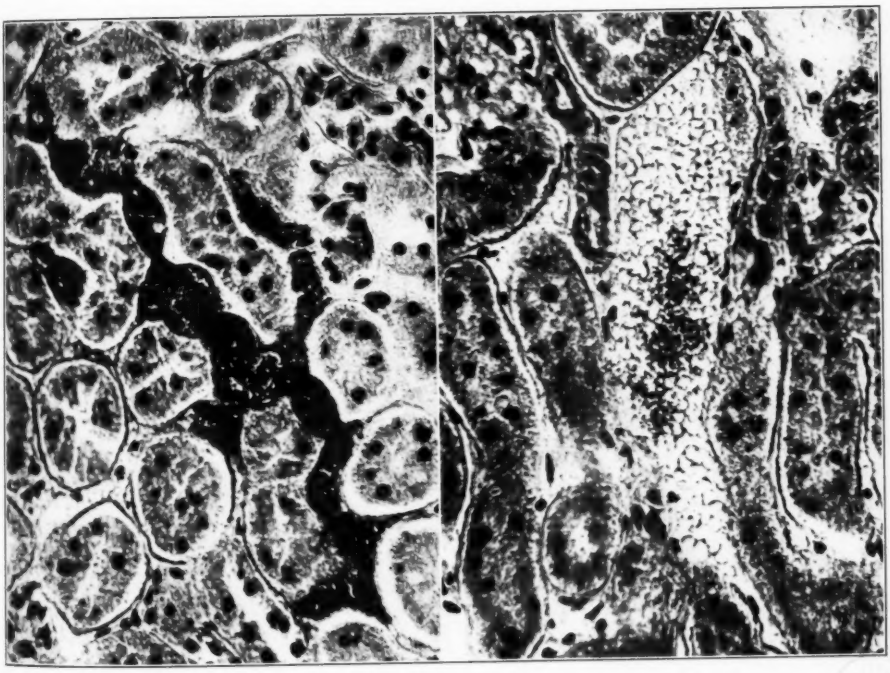

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Book

Permeability of Kidney to Bacteria

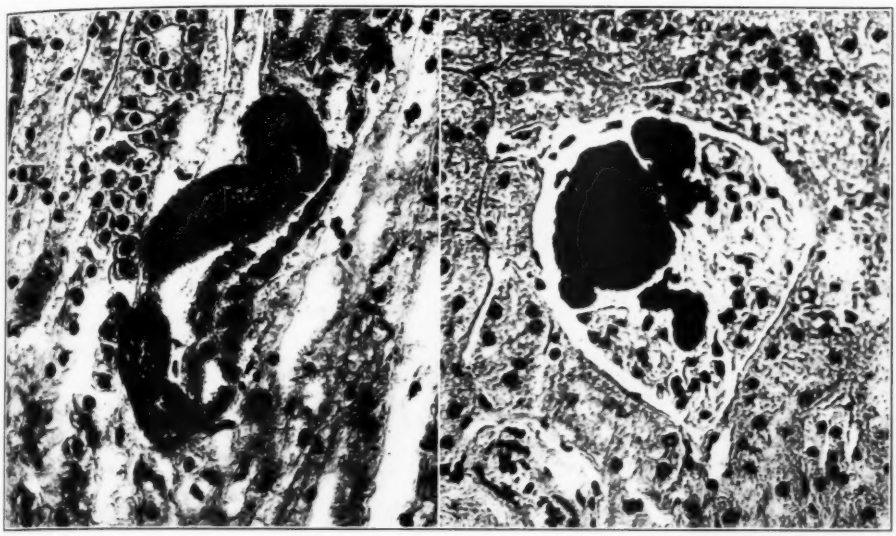
PLATE 96

FIG. 5. A colony of *B. coli* in the medulla. × 410.

FIG. 6. A colony of staphylococci in a glomerulus. × 410.

FIG. 7. A colony of staphylococci in the medulla. × 410.

FIG. 8. Staphylococci within the lumens of tubules. × 410.



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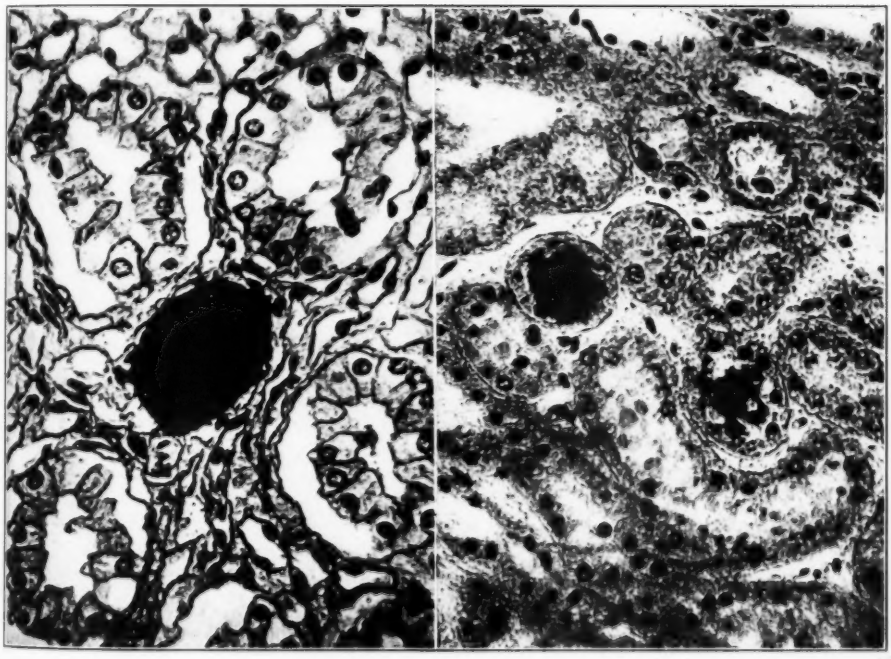

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PLATE 97

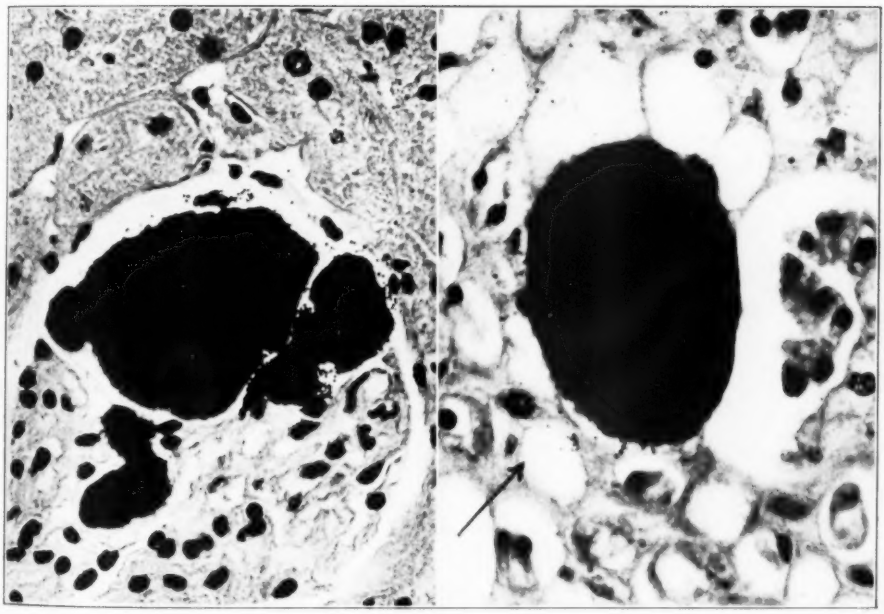
FIG. 9. A developing abscess, due to *Staphylococcus aureus*. × 100.

FIG. 10. Part of the same glomerulus shown in Fig. 6, to illustrate the shifting of bacteria in sections. × 900.

FIG. 11. A colony of staphylococci in the medulla. × 900.

The left and upper borders of the colony are not in focus in the photograph, but are seen to be quite sharp and well defined on direct examination with the microscope.

9



10 11

THE MICROINCINERATION OF HERPETIC INTRANUCLEAR INCLUSIONS *

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In view of the frequent association of inclusion bodies with virus infections any knowledge that can be accumulated as to the nature of these bodies will be of value in the investigation of the etiological agents. With this in view a study of the inorganic residue of the inclusion bodies associated with the virus of herpes simplex was undertaken. The technique of microincineration as first devised by Policard [1] and later modified by Scott [2] was employed.

Scott [3] in investigating the ash content of the nuclear inclusions of the submaxillary gland disease of guinea pigs found that they left little or no ash after incineration. Cowdry [4] obtained similar results in his investigation of the nuclear inclusions produced by the virus of yellow fever. On the other hand, Covell and Danks [5] observed an abundant, compact ash content in the cytoplasmic inclusions of rabies. Danks [6] found a definite ash content in the residue of incinerated Borrel bodies of fowl-pox, also a cytoplasmic inclusion. These are the only inclusion bodies so far examined by the technique of microincineration, which leaves open the interesting question as to whether or not the study of the remaining inclusions will disclose a tendency for nuclear inclusions to show a relative absence of ash and for cytoplasmic inclusions to exhibit very much ash.

Material and Methods

The herpetic virus † was injected into rabbits under ether anesthesia by the intracerebral route in the usual way. The animals were sacrificed at various stages in the resultant reaction. Portions of their brains were immediately removed and fixed for 24 hours in a mixture of 9 parts of absolute alcohol and 1 part of neutral formalin. They were then passed through 4 or 5 changes of absolute alcohol at

* Aided by a grant from the Rockefeller Foundation for research in virus diseases.
† Obtained through the courtesy of Dr. T. M. Rivers.
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intervals of 1½ to 2 hours, placed in a solution of equal parts absolute alcohol and xylol for ½ hour, cleared in xylol for 12 hours, and embedded in paraffin.

Serial sections, 3 microns thick, were made from the left cerebral cortex near the site of injection. Adjacent sections were alternated on two slides, one slide for staining and the other for microincineration. Those for incineration were mounted with absolute alcohol and placed in an electric quartz oven, the temperature of which was gradually increased over a period of 35 to 40 minutes to approximately 620° C. The slides were then cooled, covered with No. 0 coverslips and sealed with paraffin. The control sections were mounted with egg albumin (3 drops of a mixture of equal parts egg albumin and glycerin in 50 cc. of water) and stained with Giemsa's stain.

The microscopic equipment and illumination for examination of the incinerated sections were the same as used by Cowdry [4] in the histochemical investigation of the intranuclear inclusions of yellow fever. For simultaneous examination of the corresponding stained sections a Spencer monocular microscope, objective 1.8 mm. N.A. 1.25, equipped with an Abbé condenser was used. An iris diaphragm and 10 x and 20 x oculars were employed in the study of both stained and incinerated sections.

Observations

It was apparent from the start that the general topography of the incinerated and stained slides was remarkably similar. However, the striking difference in the stained slide between the normal nuclei and those containing inclusions was not so marked in the incinerated slide. For this reason positive identification of the same cell in both slides was necessary. When the cellular ash on the incinerated slide could not be recognized as identical with the cell containing the inclusion observed in the stained slide, it was not considered.

Insistence on this positive identification necessitated study of inclusions that were near such conspicuous landmarks as arteries and veins. The use of sections 4 and 5 microns thick resulted in so changing the configuration of the field as to throw doubt on the identification of a single cell on the two slides. It is for this reason that sections were cut at 3 microns as specified. By the alternate use

of incinerated and control sections it was then feasible to locate the ash on the incinerated section just 3 microns beyond the level of the stained sections, making possible the observation of the same inclusion in both slides. In many instances a single nucleus containing an inclusion could be located in three consecutive sections, for example, in two stained sections and in the intervening incinerated section.

The accompanying plate has been divided into two parts, left and right, to facilitate comparison of sections of the same nuclei as they appear in stained and incinerated preparations. Thus on the left are shown drawings of inclusion-containing nuclei stained with Giemsa and on the right, with the corresponding numbers, are shown drawings of adjacent sections of the same nuclei after microincineration. Nuclei of approximately the same size were selected and grouped to illustrate the contrast between young and old inclusions (Lauda and Luger [7]). Younger inclusions than shown here may be found, but because they contain considerable basophilic chromatin which is known to leave a definite ash it would be impossible in the presence of this chromatin to determine how much, if any, of the observed ash was due to inclusion material. Only those inclusions, therefore, that exhibited no visible basophilic chromatin in their substance were included. When the inclusions had progressed to the stage that the nuclear membranes were no longer intact it not only became difficult to identify them after incineration but it was felt that the conditions would scarcely be typical.

In order to conserve space on the plate the cytoplasm has been omitted. Figures 1–4 show nuclei containing typical young inclusions that exhibit a marked contrast to the more advanced inclusions (Figs. 5–12) characterized by an increase in the halo and diminution of inclusion content. It is obvious that the younger inclusions exhibit a more abundant ash; in fact a definite residue is often difficult to demonstrate in the old inclusions (Figs. 10 and 12).

A study of the stained sections reveals an absence of any structure resembling a nucleolus, the disintegration of which has been previously described (Goodpasture [8]). Nucleoli of normal cells of the type studied have a definite ash content unmistakable in configuration; such a body is not observed in the incinerated inclusion-containing nucleus.

Concentrated at the nuclear membrane are the basophilic masses that many investigators believe represent the chromatin material seen in the body of the younger inclusions. On the incinerated slide these masses leave a compact white ash.

Discussion

The striking finding of this investigation is that as the inclusion ages the inorganic content diminishes. If one assumes that the inclusion represents a cellular alteration due to a physical or chemical influence, the presence or absence of ash would merely represent the acquisition and retention of inorganic constituents of metabolism. This might easily be the result of a change in the permeability of the nuclear membrane. Such hypothetical alteration is consistent with the diminution of the ash in old inclusions of herpes simplex, for, as is well known, the nuclear membranes about the very old inclusions disappear. Another possible explanation for this phenomenon may lie in a conversion of the inorganic content from a comparatively non-dialyzable form in young inclusions to a highly dialyzable form as the inclusion ages. Lauda and Luger [7] have demonstrated that the nuclear membrane undergoes a degeneration in herpes simplex, even to the extent of obliteration. Therefore, it would seem likely that the change in inorganic content is due to a disturbance of the function of the membrane.

As previously explained, it was deemed advisable to minimize a gross source of error by limiting the observations to nuclei containing no visible basophilic chromatin in the inclusion body. Even this precaution does not guarantee the absence of basophilic chromatin and it is quite likely that the heavier ash of the younger inclusions may be due to chromatin that has not migrated to the periphery of the nucleus.

The investigations of Scott [3] and Cowdry,[4] on the submaxillary gland disease of guinea pigs and on yellow fever respectively, have disclosed a slight to doubtful ash content for the nuclear inclusions concerned, while Covell and Danks [5] and Danks,[6] working in the same laboratory and using the same technique, investigated rabies and fowl-pox respectively and found an abundant ash residue for the cytoplasmic inclusions. The finding of little or no ash residue in the mature herpes simplex inclusions is consistent with the generaliza-

tion that the nuclear inclusions examined by microincineration differ from the cytoplasmic ones in this respect.

If one assumes that the inclusion contains the etiological agent, be it organismal or inanimate in nature, it must be granted, in the light of studies thus far made, that either the inorganic content of the etiological agent varies from the great amount found in cytoplasmic inclusions to practically *nil* in intranuclear inclusions, or that the changes in inorganic material are the results of cellular changes aroused by the presence of the etiological agent. On the other hand, the difference in the inorganic residue may have no relation whatever to the specific etiological agents, for these agents may be merely acting at the interface between the cell membrane and the surrounding tissue or between the nuclear membrane and the cytoplasm.

Summary

Microincineration of herpetic intranuclear inclusions from the cerebral cortex of rabbits reveals the presence of considerable inorganic material in young full inclusions with a progressive decrease in amount as the inclusions develop. Mature inclusions are frequently devoid of any inorganic residue.

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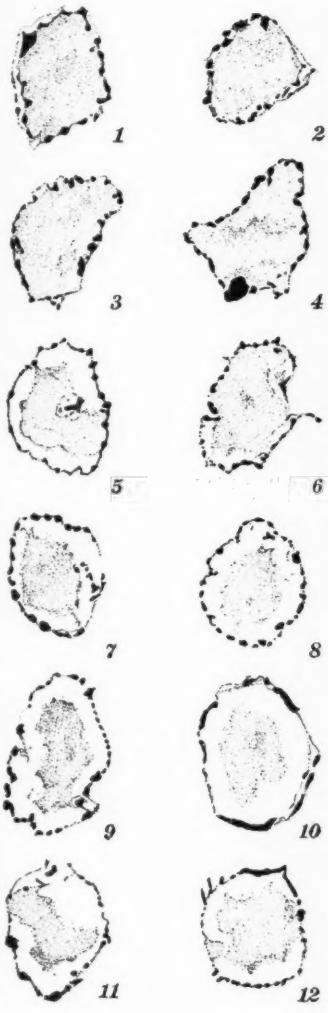
DESCRIPTION OF PLATE

PLATE 98

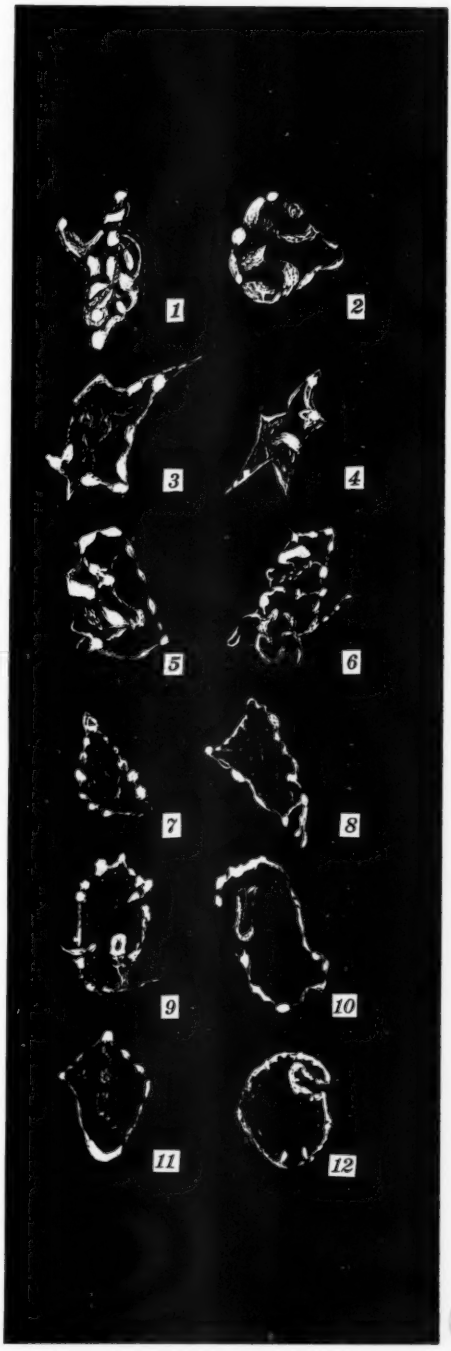
The left half of the plate contains drawings of cortical cells of rabbit made from stained sections and on the right half, numbered correspondingly, are drawings of the same nuclei made from adjacent 3 micron sections after micro-incineration. The cytoplasm has been omitted to save space.

FIGS. 1 to 4 inclusive. Drawings of stained and incinerated nuclei showing young inclusions of herpes simplex. Note the presence of ash throughout the nucleus.

FIGS. 5 to 12 inclusive. Drawings of stained and incinerated nuclei showing more advanced stages of inclusions of herpes simplex in varying degrees of advancement. Note the steady diminution in ash as the halo develops. The minute amounts of ash seen in Figs. 7 to 12 are typical of mature inclusions.



Rector and Rector



Microincineration of Herpetic Inclusions

RARE FORM OF SACCULAR CARDIAC ANEURYSM WITH SPONTANEOUS RUPTURE *

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A not uncommon late complication of coronary arterial disease and cardiac infarction is ventricular aneurysm, which rarely ruptures except in the presence of reinfarction of the old scar or adjacent myocardium. Commonly such aneurysms develop only at the site of large and healed infarcts, are of considerable size and have a wide communication with the cavity of the chamber involved. Actually many of them represent merely a dilatation of the heart wall rather than aneurysm in the more restricted sense of the term.

Among a series of 40 cases of spontaneous rupture of the heart reported by us in a previous paper [1] there was encountered one example of rhexis of a true saccular aneurysm communicating with the left ventricular cavity by only a small opening, so that the aneurysm lay over the external surface of the heart. In view of the unique character of the lesion, our failure to encounter an identical example among the exhaustive literature dealing with cardiac rupture, and because the length of the first communication necessitated only a brief consideration of individual instances, we have felt it advisable to make a more detailed account of this particular case

A similar aneurysm which, however, failed to rupture was described by Corvisart [2] in 1797. It occurred in a negro 27 years of age who entered the hospital "in inexpressible anguish and anxiety; breathing was laborious and interrupted; he suffered little pain in the thorax, which besides, sounded well in its whole extent; he complained of feeling a violent pain both toward the region of the stomach and of the liver." Death occurred the following day. The heart was reported to be of natural size, "but the superior and lateral portion of the left ventricle was surmounted by a tumor almost as large as the heart itself." This tumor was of cartilaginous consistence but of the appearance of muscle, communicated with the ventricle by a small opening, and contained coagula.

* Received for publication May 22, 1933.

Report of Case

Clinical History: W. E. J., Male, aged 45, a lieutenant in the Portland fire department, died suddenly while driving his car. An autopsy was made at the direction of the coroner. Three months prior to death the man had consulted a physician because of severe pain in the stomach, radiating to the left arm, pain in the back and headache. On this occasion he appeared very ill and suffered intensely, requiring a half grain of morphine hypodermically for relief. The heart was irregular, the valve tones indistinct, the pulse feeble and the arteries were inelastic to palpation. The systolic blood pressure was recorded as 160 but there is no notation of the diastolic reading. A hasty urinalysis revealed a trace of albumin but nothing else of consequence. The past history was irrelevant except for loss of appetite and headache extending over a period of five years. In early life he drank to a moderate extent but for the past few years had indulged but very little. On the day of death he was on duty and feeling unusually well.

Postmortem Examination *

For the sake of brevity only the more pertinent findings will be described.

Even before opening the pericardial sac the presence of hemorrhage was anticipated by the dark color and tenseness of the membrane. Upon incising the pericardium the sac was found to be filled with blackish clotted blood, obscuring from view the empty and contracted heart and forming a cast about it. When the clot was removed there was seen over the anterolateral aspect of the left ventricle an area of fibrous adhesions 7.8 by 4.5 cm. in extent binding the epicardium and pericardium. Similar bands obliterated the pericardial space over the surface of a mushroom-like mass situated well up toward the atrioventricular sulcus and between the anterior interventricular and circumflex branches of the left coronary artery (Fig. 1–A). Along the inferior border of this pouch and 1 cm. lateral to a line drawn through its center was a slit-like, ragged-edged opening 4 mm. in length. A probe passed into this defect led through the 1 mm. wall at the base of the aneurysm into its cavity which lay over the external surface of the left ventricle. On separating the patch of pericardial adhesions lying inferior to the aneurysm a layer of clotted blood was exposed in the fibrous tissue holding the pericardia together. Tracing the hemorrhage cephalad one found it leading to the rent in the inferior wall of the aneurysm mentioned previously. It would thus appear that rupture took place at this

* Path. No. 10,725, Group F, Case No. 35 (see Ref. 1).

point and that the escaping blood then dissected through the adhesions for some distance before finally breaking out into the unobliterated portion of the pericardial sac (Fig. 1–A). Further fibrous bands obliterated the pericardial space over the anterior and left aspects of the left atrium and the greater part of the posterior surface of the left ventricle, stopping abruptly 2 cm. from the interventricular septum posteriorly and 2.5 cm. from the apex inferiorly. In addition to dissection along the course already described the extravasated blood had also made its way along the base of the aneurysm over the surface of the left atrium as far as the point of entrance of the left pulmonary veins and into the space separating the root of the aorta and pulmonary artery, tearing adhesions wherever these existed along its path.

The aneurysm measured 4 by 4 by 3 cm., the last mentioned figure being the height above the surface of the heart. The wall varied between 0.1 and 0.7 cm. in thickness and appeared to consist solely of pericardium, fibrous connective tissue, epicardium and possibly endocardium, bound intimately together. The lining of the sac was generally smooth and whitish, except for linear grooves and ridges radiating outward in a fan-like fashion from an opening measuring 0.5 by 0.3 cm., located at the base of the cavity and leading directly through the myocardium into the left ventricular chamber. In several places the smooth and glistening white lining of the pouch was coated with a thin, yellowish brown film of fibrin; otherwise the aneurysm was empty.

An incision carried through the opening leading through the left ventricular wall disclosed patchy, whitish scar tissue in the myocardium immediately around the defect and for approximately 2.5 cm. outward into the muscle on all sides of it (Fig. 1–B). This was the only macroscopic lesion in any part of the heart muscle. Moreover, after sectioning it became evident that the fluted ridges around the margins of the opening into the aneurysmal sac were directly continuous with several greatly attenuated trabeculae carneae. Elsewhere these muscle bands were of average normal size except along the septal surface of the ventricle where there was some flattening and atrophy. The internal end of the communication between the ventricle and sac on its surface was situated 1.5 cm. from the attachment of the posterior mitral valve cusp to the mitral ring. The anterior and posterior walls of the left ventricle averaged

2 cm. in thickness, decreasing to 1.5 cm. at the point of the opening leading to the aneurysm and to 1 cm. at the apex of the chamber. The several valves of the heart and the endocardium of the different chambers displayed no evidences of disease.

The ostium of the right coronary artery appeared almost closed by sclerosis, while that of the left was greatly narrowed by the same process. Extending cephalad from the commissure separating the anterior and left posterior aortic leaflets were several shallow linear wrinkles in the intima but nothing further suggestive of syphilis was observed at any other point along the course of the aorta. Aside from the plaques obstructing the coronary orifices there were a number of small and beaded yellowish streaks in the intima of the sinuses of Valsalva. Similar plaques were scattered along the intima of the thoracic aorta.

The main left coronary artery was a large and freely patent vessel exhibiting only small atheromatous plaques. Its anterior interventricular branch was likewise of ample size, but in places the lumen was moderately obstructed by whitish sclerotic patches. The same was true for the circumflex division and the right coronary artery. The latter vessel did not extend for any appreciable distance past the margo acutis, and to compensate for this the left circumflex gave off a posterior interventricular branch which coursed toward the apex and formed an adequate anastomosis with the anterior descending artery. At the point of bifurcation of the left coronary there originated two good sized twigs which supplied the portion of the left ventricle where the aneurysm and myofibrosis were located. The smaller of these passed directly to the area of scarring about the opening leading through the ventricular wall, and macroscopically its lumen at one point seemed to be occluded by an old and organized thrombus. No fresh thrombi were discovered in any of the coronary vessels.

The heart, together with the adherent portion of the pericardium and ascending aorta, weighed 640 gm. The kidneys had a combined weight of 440 gm. The capsule of each stripped readily, revealing a faintly and finely granular surface. On section the cortex appeared to be of normal thickness and the rays and labyrinths were everywhere plainly outlined. The lungs, spleen and liver displayed nothing other than acute passive hyperemia.

Microscopic Examination

Heart: Sections from blocks cut to include the opening in the left ventricular wall and the adjacent myocardium for 2.5 cm. on either side of it reveal irregular patches of connective tissue interrupting the muscle substance (Fig. 1–B). For 5 to 7 mm. around the margins of the hole the tissue is almost wholly fibrous but becomes progressively less so farther away from the defect. Several of the trabeculae carneae in this region are reduced to dense scar tissue or show only occasional persistent muscle cells. Between two of these muscles small thrombi consisting of fibrin and leukocytes are discovered. Near the opening the fibrous connective tissue nuclei are larger and more numerous than elsewhere and yet are widely separated by an abundance of coarse and compact intercellular collagen, among which is a fair number of thin-walled and engorged blood vessels. More peripherally the vessels are larger and possess a definite muscularis. None are occluded by thrombi nor do they exhibit evidences of arteriolosclerosis. Approaching the margin of the aneurysmal opening one finds the scar replacing the myocardium passing uninterruptedly from the sac on the external aspect of the heart along the defect to become continuous with the endocardium. At the junction of the scar and endocardium there is edema, engorgement of capillaries and hemorrhage by diapedesis. The connective tissue cells forming the immediate lining of the communication uniting the ventricular cavity and the aneurysm have very large and hyperchromatic nuclei whose long diameter lies parallel to the opening and at a right angle to the myocardial cells. In this location the tissue is compact rather than loose, possibly indicating a compression by the pulsation of the blood passing through the opening. A true endothelial lining is lacking but the inner surface is nevertheless smooth and not covered by thrombi. As in other parts of the scar there are moderate numbers of capillaries about which are lymphocytes, plasma cells and histiocytes.

In the wedge-shaped area between the epicardium and base of the aneurysm (see Fig. 1–B) there is a rich fibroblastic proliferation and the whole zone consists of these cells and histiocytes. The muscle cells adjacent to the epicardium are hypertrophic, as evidenced by their great size and large hyperchromatic nuclei. Quite generally the muscle fibers are separated by very loose and apparently edematous

connective tissue. A deposit of fibrin covers the epicardium along this angle.

Sections from a number of different parts of the aneurysm wall reveal the lining to be undulating, interrupted by occasional sharp clefts, and consisting of compressed fibrous connective tissue, at times coated with a thin layer of fibrin or blood platelets (Fig. 2). Nothing resembling normal endocardium is identified. Passing from within outward there is encountered first a zone of solid connective tissue with abundant intercellular substance, followed by loose edematous tissue of the same nature containing numerous but irregularly distributed capillaries distended with blood and sometimes surrounded by fresh hemorrhage. At least one of these vessels exhibits a definite rupture of its wall, indicating that in this instance at least the surrounding hemorrhage is due to rhexis. Next there is a very active fibroblastic zone displacing almost entirely the fat cells of the subepicardial region. The blood vessels here are distended and collared by numerous lymphocytes, plasma cells, histiocytes, a sparse sprinkling of neutrophilic polymorphonuclear leukocytes and multinucleated giant cells engorged with yellowish blood pigment. The pericardium and epicardium are so intimately adherent that it is practically impossible to distinguish one from the other. Two coronary arterioles included in the subepicardium display proliferation of the intimal cells with resultant narrowing of their lumens.

Still another section, coming from the vicinity of the rupture through the aneurysm wall, discloses extensive recent hemorrhage into the fibrous adhesions between epicardium and pericardium and probably represents another ramification of blood from the point of rupture. More inferiorly and toward the apex of the ventricle the hemorrhage spreads out extensively and diffusely, breaking up and widely separating the adhesions. By these routes the dissecting hemorrhage must eventually have reached the nonobliterated portion of the pericardial sac and led to fatal hemopericardium.

The main left anterior interventricular coronary artery is the seat of intimal atherosclerosis, which has produced eccentric narrowing of the lumen. The deeper and more external parts of the plaques are often the seat of fatty degeneration and contain dust-like particles of lime salt. There are no apparent breaks in the endothelium. Lying free within the lumen and only partially occluding it is a small

ante mortem clot consisting of fibrin and leukocytes. The media is thin and atrophic while the adventitia is unaltered.

Microsections from three different points along the course of the small artery originating at the bifurcation of the main left coronary artery and coursing directly toward the scar about the aneurysm opening disclose advanced atherosclerosis of the intima with pronounced narrowing and eccentric displacement of the lumen. Thrombosis is lacking, although a few scattered erythrocytes and leukocytes are present. The deeper parts of the various patches of sclerosis are fatty, containing fat-laden phagocytes and large plates of calcium, not infrequently encased by multinucleated foreign body giant cells. Also there are several endothelial-lined, blood-containing channels in the degenerated parts of the plaques, but nothing whatsoever to indicate the existence of an organized and recanalized thrombus. The media of this vessel is thin and in places infiltrated with lymphocytes and plasma cells, yet without evidence of necrosis or scarring such as might be anticipated in chronic syphilis. Similar but larger cell collections collar the adventitial vessels in which evidences of endarteritis are lacking.

Microscopically the right coronary artery displays the same atherosclerotic process present in the other arteries with the additional finding of atheromatous ulceration. Although there is stenosis the lumen is not thrombosed. Perivascular accumulations are lacking.

The histopathological changes in the aorta are quite the same as those described for the coronary vessels, namely, varying degrees of intimal atheroma with softening, collars of lymphocytes and plasma cells about the vasa vasorum of the media and adventitia, sometimes with minute flame-like scars but never obliterative endarteritis.

Kidneys: A microscopic study of these organs reveals occasional completely hyalinized glomeruli, hyperemia of all other glomerular tufts, and subintimal hyaline thickening of certain intertubular and afferent glomerular arterioles, leading in some instances to moderate stenosis.

Pathological Diagnoses: Arteriosclerotic narrowing of coronary arteries, especially pronounced in one branch of the left; patchy myofibrosis of left ventricle with formation of saccular aneurysm, spontaneous rupture of this aneurysm and fatal hemopericardium; focal

fibrous pericarditis; atherosclerosis of aorta; possible rheumatic arteritis and aortitis; hypertrophy of heart; slight arteriolosclerosis of kidneys; probable hypertensive cardiovascular renal disease; generalized acute passive hyperemia.

Discussion

From the foregoing description it is clear that death was the result of spontaneous rupture of the thin and fibrous wall of a very unusual type of cardiac aneurysm and the pericardial adhesions about it. The mechanism by which the aneurysm was produced is less easily explained. Although meager, the clinical data suggest that on the one occasion, three months prior to death, when the man was seen by a physician he was suffering from cardiac symptoms referable to the heart and compatible with coronary thrombosis. The one other significant observation at this time was the systolic blood pressure of 160 recorded during the seizure. It is common knowledge that the blood pressure decreases after a coronary accident and it is probably not erroneous to postulate that prior to the attack of cardiac distress the tension was greater than 160 mm. Hg. In any event the man had hypertension during the seizure and it is unnecessary to prove that the blood pressure was ever greater. If we accept Bell and Clawson's [3] criteria for the clinical and pathological diagnosis of essential hypertension this case becomes a clear-cut instance of hypertension, for not only was the blood pressure elevated beyond the normal limit of 150 mm. of mercury during life, but at autopsy there was found cardiac hypertrophy (640 gm.) without demonstrable cause other than possibly from pericardial adhesions and the characteristic renal arteriolar lesions of essential hypertension.

Granting that primary hypertension formed the basis of the vascular disease there yet remains the problem of genesis of the aneurysm. In the absence of demonstrable coronary thrombosis one must seek to explain the myofibrosis on other grounds. Slow and progressive ischemia of a small area of myocardium brought about by high grade atherosclerosis of the vessel supplying the myocardial scar may have led to disappearance of patches of heart muscle and finally replacement fibrosis. Less probably the scar may represent the end-result of focal rheumatic or syphilitic myocarditis. Definite proof of

the existence of either of these diseases is lacking but certain features about the perivascular aortic and coronary arterial lesions are more suggestive of the former.

When and how the aneurysm formed is a question to which we can give no definite answer. In view of the clinical history there is reason to believe that it may have had its inception three months prior to death either during or shortly after the attack of severe thoracic pain and cardiac embarrassment. It seems possible in the presence of hypertension there may have been sufficient increase of intracardiac pressure exerted over the small area of already existent fibrosis to cause stretching or dissection until the external surface of the ventricle was reached, after which instead of immediate rupture the scar became adherent to the pericardium and these structures continued to hold for a time before finally giving way. Still another possibility is that of cardiac infarction, partial rupture during the acute stage of necrosis, followed by the formation of pericardial adhesions and finally rupture.

Although ordinarily cardiac aneurysm develops along the whole extent of the scar following healing of an infarct we have seen at least one striking exception to the rule. This case (No. 8370) (Fig. 3) may possibly represent an earlier stage of saccular aneurysm than the one forming the basis for our communication, and if so serves to show that not all cardiac aneurysms demand a large area of scarring for their development. In the example just mentioned a portion of a massive healed infarct involving the anterior wall of the left ventricle and interventricular septum was separated from the main cicatrix by a zone of intact myocardium and in the small part there formed a little pouch near the apex and adjacent to the interventricular septum. The peculiar and unusual feature was the fact that on either border of the wholly fibrous wall of the sac the ventricular muscle was of normal structure and thickness so that it appeared as if the aneurysm had been punched out over only a small area. As in the first case (No. 10,725) the neck of the pouch was somewhat narrower than the sac itself and the aneurysm wall externally consisted solely of fibrous tissue and epicardium. What would have been the later history of this aneurysm had the individual survived for a longer time is conjectural, but it seems possible that it might later have ballooned out over the external aspect of the ventricle as in the

first case, although the presence of the tampon thrombus that filled the sac may conceivably have organized and precluded such a complication.

Summary

A rare and almost unparalleled type of cardiac aneurysm situated over the external aspect of the left ventricle is described and the possible causes for its development are discussed.

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DESCRIPTION OF PLATES

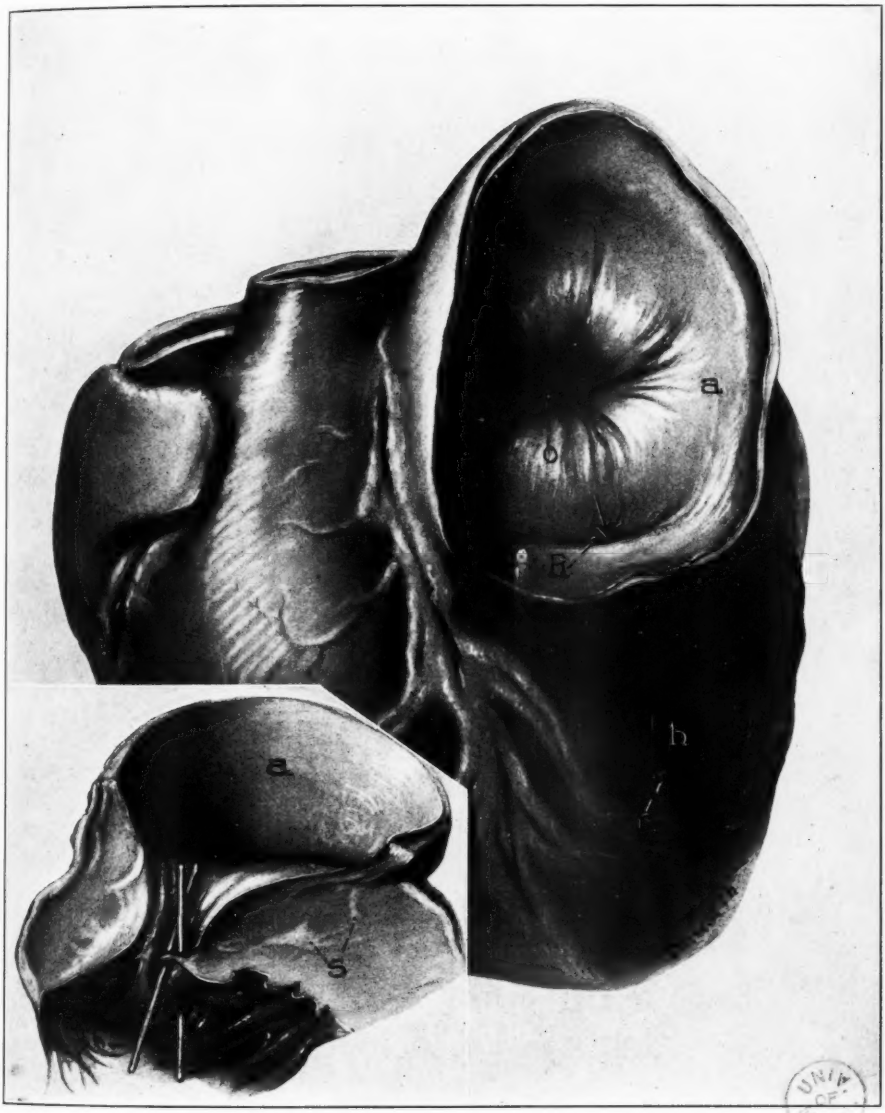
Plate 99

Fig. 1–A. Case No. 10,725. Drawing, actual size. Anterior aspect of heart showing position and appearance of saccular aneurysm after removing a portion of its wall.

a = aneurysm.
o = opening with fluted margins, leading to left ventricular cavity.
R = point where rupture began at base of aneurysm wall.
r = fresh dissecting hemorrhage in pericardial adhesions.
h = torn fibrous adhesions over epicardium.

Fig. 1–B. Actual size drawing of aneurysm and left ventricular wall after hemisection of the sac to display the size and character of the defect and structure of adjacent heart muscle.

a = aneurysm.
s = patchy myofibrosis in vicinity of opening leading to aneurysm.
m.v. = posterior cusp of mitral valve.
Marker rods indicate ramifications of the channel between the atrophic trabeculae carneae and the main communication.



I–B I–A

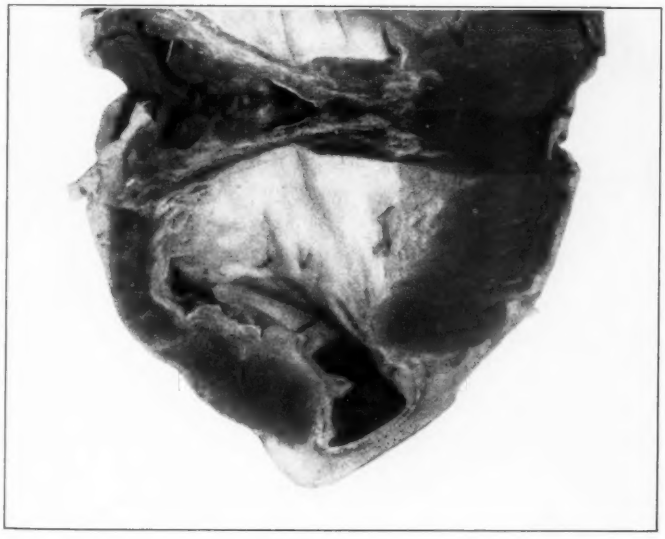
Hunter and Benson Rare Form of Saccular Cardiac Aneurysm

PLATE 100

FIG. 2. Low power photomicrograph showing entire thickness of cardiac aneurysm in case No. 10,725. To the right is seen a small portion of the undulating lining of the sac, at this point coated with fibrin. On the left border are torn vascular fibrous adhesions replacing the epicardium. All of the remainder of the wall consists of relatively young fibrous connective tissue in which a number of distended capillaries are visible. The complete absence of myocardium is evident.

FIG. 3. Case No. 8370. Photograph displaying septal surface of left ventricle. The interventricular septum (sectioned) and anterior wall of the ventricle are the seat of a healed infarct extending to the endocardium, which is greatly thickened and whitish. Near the apex is a small aneurysm surrounded by intact myocardium of normal thickness. Only the tamponing thrombus, thin fibrous wall of the sac and epicardium separate the cavity of the ventricle from the external surface of the heart.

2



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Hunter and Benson Rare Form of Saccular Cardiac Aneurysm

ACTINOBACILLOSIS OF MAN *

REPORT OF A FATAL CASE

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In 1902 Lignières and Spitz,[1] working in the Argentine, identified the *Actinobacillus* as one of the etiological agents of so-called bovine actinomycosis. They proposed the designation "Actinophytose à actinobacille" for the disease, which by subsequent investigators has been called actinobacillosis.

Clinically and pathologically bovine actinomycosis and actinobacillosis have few distinguishing characteristics. The chief point of difference is situation. Actinomycosis usually involves the jaw bones as a rarefying osteitis with formation of sinuses, whereas actinobacillosis more often attacks the soft tissues of the mouth, pharynx, tongue and skin, with secondary involvement of the adjacent lymph nodes. Notwithstanding the clinical and pathological similarities of the diseases, the causal organisms are quite distinct, except in one feature. Both have the property of evolving aggregations in the tissues and exudates known as sulphur granules, about which radiating clubs are easily identified. On crushing the granules, however, the *Actinobacillus* yields only small Gram-negative bacillary forms, whereas *Actinomyces* reveals the distinctive branched Gram-positive filaments. Lignières and Spitz found the *Actinobacillus* capable of producing an experimental disease of cattle identical with the natural infection. It possessed a variable virulence for guinea pigs and rabbits.

The conclusions of Lignières and Spitz have been confirmed repeatedly by investigators working on the continent of Europe, the British Isles and Canada. Recently Thompson [2,3] reviewed the subject, and reported for the first time the existence of actinobacillosis as a common malady of cattle in the United States. He concluded: "Actinobacillosis is common among cattle in the United States. It would seem that the condition here is similar to that in other coun-

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tries where the greater percentage of so-called bovine actinomycosis is due to *Actinobacillus lignièresi*." For more complete consideration of the subject reference may be made to Thompson's paper.

Thompson and Willius [4] in 1932 reported the identification of *Actinobacillus* bacteremia in man. The patient was subsequently observed by Lawrence, Neuhauser and Howell [5] and in a confirmatory, supplementary report it was stated by these observers that the illness had apparently terminated in recovery. Ravaut and Pinoy [6] in 1911 described the occurrence of *Actinobacillus* meningitis. Their patient likewise recovered. The present case of *Actinobacillus* infection, the third in man to be reported, was fatal; consequently it is the first in which pathological studies have been made. The presentation of this case is our objective in this paper.

Report of Case

Clinical History: A man, aged 34 years, came to The Mayo Clinic Jan. 9, 1933. He had been employed as a clerk in a clothing store until a few months before, and more recently had been engaged in selling floor wax throughout southern Minnesota. He was born in southeastern Minnesota, where he always had resided. His health had been quite normal until the summer of 1932, when he began to lose weight gradually, for no apparent reason. Periods of anorexia were sometimes experienced. All bodily functions apparently had remained normal. He had not experienced excessive thirst or polyuria. There was no cough. On Dec. 25, 1932, he had no appetite, felt poorly and that evening retired early. On December 27 he became acutely ill and went to bed. The illness was considered by the patient to be influenza; it was characterized by malaise, weakness, fever to 102° F, and profuse perspiration. About Jan. 1, 1933, severe pain developed in the thorax posteriorly between the shoulders. Plasters were applied and on January 7 the pain entirely disappeared. The illness, however, was progressive; appetite failed, weight was lost more rapidly and weakness increased to the point of exhaustion. Fever continued, and on January 8 drowsiness and mild delirium supervened.

On the patient's admission to the clinic he appeared to be acutely ill and very weak. His temperature was 102° F, pulse 116 beats and respiration 32. Rather rapid loss of weight was evident (from 185 to 135 pounds). The skin was warm and moist. The eyes were rather prominent. The tongue was coated and the pharynx hyperemic. An enlarged cervical lymph node was palpable on the right side. A friction rub was noted in the right midclavicular line at the level of the fourth rib. Resonance was impaired at the bases of both lungs and moist râles were elicited at the base of the left lung. The heart was slightly enlarged, by percussion. The abdomen was distended and held tense; peristalsis was active. Roentgenograms of the thorax were interpreted as revealing extensive pneumonic consolidation at the base of the left lung, probably with fluid. A moderate degree of anemia was present. The concentration of hemoglobin was 69 per cent, erythrocytes numbered 3,510,000 and leukocytes 7000 in each cmm. of blood. The percentages of the various types of leukocytes were as

follows: polymorphonuclear neutrophils 77, lymphocytes 12 and monocytes 11, with changes in the leukocytes indicative of severe infection. Urinalysis revealed albumin, graded 2; sugar, graded 2; hyaline casts, graded 4, and an occasional erythrocyte; acetone was not present. Urea was present in the blood in essential normal concentration (44 mg. in each 100 cc. of blood), and sugar elevated (326 mg. in each 100 cc. of blood). Agglutination reactions for typhoid, paratyphoid and undulant fevers were negative. The Wassermann reaction of the blood was negative.

The patient was placed on a diabetic regimen and with use of insulin the blood sugar was brought to within normal limits. The fever increased to 103° F, and remained elevated, except for a rather sharp fall to 98° F on the morning of January 11, with subsequent rise to 102.8° F by noon of that day. The pulse and respirations remained rapid. On the evening of January 11 his breathing became more labored and respirations markedly accelerated. He was placed in an oxygen tent but failure was progressive and rapid. Death occurred at 12.17 A.M., Jan. 12, 1933.

At the time of death the diagnosis as to type of infection still was indeterminate. In many respects the illness resembled typhoid fever, and also influenzal pneumonia. The pneumonia, however, was atypical. The severity of the illness seemed to be out of all proportion to the physical or laboratory findings. The evidence of severe toxemia did not appear to be explained by the findings noted in the thorax, or by the diabetic state. Cultures of blood, which had been taken January 11, still revealed no growth of microörganisms when death occurred. The onset of symptoms of the fatal infection was probably about Dec. 25, 1932, and the preceding loss of weight was the result of diabetes. It is also probable that the presence of diabetes accentuated the infection and accounted for its severity, with rapidly fatal termination.

Pathological Data

Throughout both lungs were multiple, firm, grayish nodules that measured from 2 to 5 mm. in diameter. The nodules were subpleural and were also within the substance of the lung. Nodules observed beneath the pleura were distinctly circumscribed and somewhat elevated. From some of them thick, yellowish pus exuded on section, whereas others were composed of grayish consolidation or caseous material. The spleen weighed 748 gm.; it was embedded in a mass of adhesions, and was composed largely of multiple, discrete, sharply circumscribed, grayish yellow lesions varying in size from 1 mm. to 1 cm. (Fig. 1). On section these lesions contained thick yellow pus or material of thick pasty consistence, which resembled

that of caseation necrosis. The liver weighed 2600 gm. The hepatic lesions were similar in appearance to those observed in the spleen; however, they were less numerous and somewhat smaller. The substance that composed these foci resembled caseation necrosis rather than pus. One very small abscess was observed in the cortex of the left kidney. Other organs appeared to be normal. The brain and spinal cord were not examined.

Microscopic Examination: Fresh tissue was fixed in Orth's fluid, embedded in paraffin and stained with hematoxylin and eosin. The lesions are essentially the same in all anatomical situations, as observed in sections of the lungs (Fig. 2), liver (Fig. 3), spleen and left kidney. They consist of focal, sharply circumscribed zones of exudation with usually advanced necrosis of the exudate centrally and granulomatous proliferative changes peripherally. Early lesions are composed almost entirely of polymorphonuclear neutrophilic leukocytes without necrosis or peripheral granulomatous reaction. The usual and typical lesion, however, consists of the three zones: (1) a central zone of necrosis composed of granular, eosin-staining acellular material in which chromatin dust-like fragments of cell nuclei persist; (2) a wide zone about the central zone of necrosis composed of polymorphonuclear neutrophilic leukocytes in which a few lymphocytes and large phagocytic mononuclear leukocytes are also found; and (3) a peripheral zone about each lesion consisting of gradually diminishing numbers of leukocytes and gradually increasing numbers of endothelioid cells, fibroblasts, and occasionally also young blood vessels. The latter area varies from lesion to lesion, so that in some it comprises only a small portion, while in others it represents a well defined zone. The three zones are not especially distinct from one another, but each appears to blend gradually with the adjacent one. No further encapsulation exists, but adjacent to each lesion there is atrophy of parenchymal cells together with retrogressive cellular changes. Around some of the pulmonary lesions, however, there is an organizing pneumonic process for a considerable distance. In the peripheral portions of the lesions large compact aggregations of bacteria in colony formation are numerous. These masses are composed of faintly stained, small Gram-negative bacillary forms with a tendency to bipolar staining. Single organisms in the tissue are difficult to find, but a few are seen as plump, Gram-negative rods. They are not acid-fast.

Bacteriological Data

In a culture from the blood, made 24 hours before death, Gram-negative, rod-shaped forms appeared in the broth after 2 days. At the same time, a few colonies of similar organisms appeared in the agar plates. There was less than one colony for each cc. of blood used. At autopsy cultures were obtained from the blood, spleen, liver and lung. From all of these sources organisms were obtained having the following characteristics: Medium sized, Gram-negative, non-motile rods, occurring singly or in short chains in broth cultures. On blood agar, after 24 to 48 hours, the colonies were 1 to 2 mm. in diameter, round, convex and milky white. There was no effect on the blood. On nutrient agar the colonies were similar but smaller. In nutrient broth a pellicle and sediment were formed. Ehrlich's test for indol was positive after 3 days. On potato slants there was no visible growth after 6 days. Blood serum and gelatin were not liquefied after 15 days. On glycerin agar the growth was white, shiny, flat and membranous after 5 days. In sugar fermentations acid but no gas was produced in glucose, maltose, mannite and salicin. Neither acid nor gas was produced in lactose, saccharose, dulcitol and inulin. The cultural characteristics are summarized in Table I, in which the organism isolated from the present case is designated by the letter Z. For comparison the table includes the strain of *Actinobacillus* previously isolated from a human being, and in addition, typical strains of *Bacillus whitmori*, *Pfeifferella mallei* and *Actinobacillus* of bovine origin. Thompson [7] has recently recommended that these organisms be included in the genus *Actinobacillus*.

The serological relationship of the organism was determined by immunizing rabbits to produce an agglutinating serum of high titer. Similar serums were prepared with a culture of *Actinobacillus* of bovine origin and a culture of *Pfeifferella mallei*. The results of cross agglutination tests are shown in Table II. Serum Z is the one prepared with the organism isolated from the present case. Table II also includes, for comparison, the results obtained with an organism from a previous case and with a culture of *Bacillus whitmori*. Attention is called to the fact that all of these serums give a positive complement fixation test with an antigen prepared from *Pfeifferella mallei*. Although it does not appear in the table, tests were made

TABLE I

Cultural Characteristics of Genus Actinobacillus

Culture	Motility	Glycerine agar	Gelatine	Blood serum	Potato	Indol	Sugar fermentation							
							Glucose	Maltose	Mannite	Lactose	Saccharose	Dulcitol	Inulin	Salicin
1*	−	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A**	A	A	A	A	o	o	o
99†	+	Wrinkled, yellow	Liquefied	Liquefied	Growth good, yellow	−	A	o	Sl. A	Sl. A	o	o	o	o
PD‡	−	Smooth, yellow	Not liquefied	Not liquefied	Growth good, yellow	Sl. +	o	o	o	o	o	o	o	o
M§	−	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	Sl. A	o	Sl. A	o	Sl. A	o	o	o
Z§	−	Smooth, white	Not liquefied	Not liquefied	Growth poor	+	A	A	A	o	o	o	o	A

* 1 = *Actinobacillus* (bovine)
** A = Acid
† 99 = *Bacillus whitmori*
‡ PD = *Pfeifferella mallei*
§ M and Z = *Actinobacillus* (human strains)

which showed that serum Z did not agglutinate *Alcaligenes abortus* and *Pasteurella tularensis*.

To summarize the information contained in the tables it may be said that the organism under consideration shows a group relationship with *Pfeifferella mallei* and with *Bacillus whitmori*. The latter organism is the cause of a glanders-like disease in southeastern Asia called melioidosis. It also resembles more strongly *Actinobacillus*

TABLE II

Serological Reactions of Actinobacillus

Cultures	Rabbit serums prepared with culture					Complement fixation with mallei antigen
	1	99	PD	M	Z	
1*	10,240	0	20	40	160	44400
99†	0	2560	40	0	0	44444
PD‡	20	2560	2560	20	20	44444
M§	0	80	0	320		44400
Z§	80		80		5120	44400

* 1 = *Actinobacillus* (bovine)
† 99 = *Bacillus whitmori*
‡ PD = *Pfeifferella mallei*
§ M and Z = *Actinobacillus* (human strains)

lignièresi, the cause of bovine actinobacillosis, but differs from the latter in certain sugar fermentations. From the study of two strains obtained from human beings it seems most logical to consider these organisms as *Actinobacillus lignièresi*, which show slight cultural and antigenic differences from the strains of bovine origin.

It is possible that infections due to *Actinobacillus lignièresi* have not been recognized in the past, owing to the superficial similarity of this organism to those of the coli group. All Gram-negative, nonmotile, aerobic organisms that produce only acid in sugar mediums should be investigated more fully as to cultural and serological reactions.

Experimental Data

Triturated preparations obtained from the spleen, liver and lung of the patient at autopsy, ante mortem blood, and pure cultures as recovered from the ante mortem blood culture and from the lesions at autopsy, were proved to be pathogenic for rabbits, guinea pigs and mice. In these animals granulomatous abscesses and tubercle-like granulomas were evolved. The granulomatous features in the lesions were frequently much more prominent than they were in the lesions of human beings; nevertheless, they were similar if not identical with one another. A clearly evident Strauss reaction was never elicited, although in two guinea pigs mild periorchitis was revealed at autopsy. Bacterial masses in ball-like colony formation were found in many of the infectious foci of the experimental animals, similar in appearance to those that were seen in the lesions of human beings. Well defined peripheral clubs were not associated with these colony formations, as in bovine actinobacillosis, although suggestions of early club formations were sometimes observed. In coverslip preparations of the exudates the organisms appeared usually as single, plump, faintly stained Gram-negative rods, sometimes in pairs or in short filaments, many revealing bipolar staining characteristics.

The lesions in guinea pigs and rabbits also, in a general way, resembled those described for experimental glanders. The experimental disease produced by the organism from our case was chiefly distinguished from experimental glanders by failure to produce a characteristic Strauss reaction in guinea pigs, the colonization of the bacteria in aggregations in the lesions and by revealing greater pathogenicity for rabbits than for guinea pigs.

The strain of *Actinobacillus* from our case was of greater pathogenicity than generally has been described for the *Actinobacillus* of bovine origin. Lignières and Spitz [8, 9] found that their cultures had variable degrees of virulence for bovines as well as for small laboratory animals, although they distinctly described the evolution of fatal peritonitis on intraperitoneal inoculation of guinea pigs, with death of the pigs in from 12 to 24 hours when large doses were used, and in from 5 to 7 days when smaller doses were used. The character of the peritonitis produced in their animals was apparently comparable with that revealed by ours. The suggestion of a mild Strauss reaction in two of our animals is also in accord with their description

of periorchitis, which developed in guinea pigs inoculated intraperitoneally, although this reaction in their animals was of much greater severity. In the lesions of guinea pigs inoculated intraperitoneally Lignières and Spitz found the tufts or aggregations of microörganisms with peripheral clubs similar, although less distinct, to those evolved in bovine lesions. In our experimental guinea pigs, especially those inoculated intraperitoneally, the aggregations of bacteria in compact colony formation were very prominent in many of the lesions, although the zone of peripheral clubs was either only faintly suggestive or not revealed. Neither Magnusson [10] nor Griffith [11] was able to confirm this observation of Lignières and Spitz. The formation of subcutaneous abscesses in guinea pigs, which developed on subcutaneous introduction of the organisms such as we describe, was also produced by bovine strains in the experiments of other investigators.

Rabbits inoculated with the strain from our case were more readily infected than other observers have found them to be when working with bovine strains of the organism. There is perhaps no record of the production of lesions by the *Actinobacillus* in rabbits comparable to those exhibited by our animals. It is therefore apparent that in this instance we have a strain of virulence of high degree, which may account in part for the fulminating infection of our patient and also for the ease with which lesions were evolved in animals. In the case of *Actinobacillus* bacteremia studied by Thompson and Willius the organism was much less virulent for both man and laboratory animals. This is what one would usually expect, judging from the experience of other observers in working with bovine strains.

Rabbits: These animals appeared to be most susceptible to the infection (Table III). In Rabbit 1, which received an intravenous inoculation of 1 cc. of a 24 hour pure brain broth culture, the greatest virulence of the organism was manifest, death of the animal resulting in 29 hours. At autopsy small, scarcely visible, granulomatous foci were found in the liver, lungs and spleen. Microscopically these consist of focal areas of necrosis with early polymorphonuclear leukocytic exudation and beginning proliferation of endothelioid cells. The lesions are discrete, multiple and particularly prominent in the liver and spleen. Rabbits 2, 3 and 4, which received 0.3, 0.8 and 1.5 cc. of a pure 24 hour brain broth culture intravenously, lived 15, 11 and 5 days respectively. Clinically, all of these rabbits revealed

a slowly developing but progressive general reaction in which loss of appetite, emaciation and listlessness were the chief symptoms. At autopsy lesions were found similar to those seen in Rabbit 1, but more advanced. In each of the animals lesions were observed in the liver, lungs and spleen. In Rabbits 2 and 3 the kidneys also were involved, and in Rabbit 2 the cecum. In Rabbits 2 and 3 the lesions were particularly well defined. Grossly in each instance the spleens were enlarged to approximately five times normal size and were mottled by grayish areas of necrosis, sometimes discrete, but due to the enormous number of lesions becoming confluent (Fig. 4). In the livers and lungs the lesions were extremely small and appeared as a fine grayish white mottling. In the kidneys and cecum larger grayish necrotic foci appeared. Microscopically there is considerable variation in the appearance of the lesions, but they are still compatible with stages of the same granulomatous process. Briefly stated, the lesions consist of a central zone of necrosis in which nuclear detritus is rather prominent. Surrounding this polymorphonuclear leukocytes have collected and peripherally a rather well defined zone of endothelioid cells is observed. In some instances the outer portion of this area is composed mostly of fibroblasts. Interspersed with the endothelioid cells and fibroblasts are a few polymorphonuclear leukocytes, lymphocytes, mononuclear leukocytes and plasma cells. The zone of necrosis varies so that in some instances it occupies almost completely the entire focus with just a narrow rim of cellular elements, whereas in other lesions it is extremely small or almost absent. When the latter prevails the center of the lesion is either occupied by polymorphonuclear leukocytes as the predominating cell, or the entire structure is evolved as a tubercle, consisting almost entirely of endothelioid cells. Occasionally Langhans' giant cells are observed in the peripheral granulomatous zone. Bacterial colonies are rarely identified with the lesions in rabbits, but when found, as in Rabbit 4, they appear to occupy the central zone and are surrounded by an area of necrosis in which a few polymorphonuclear leukocytes are visible. The bacterial colonies are composed entirely of small, Gram-negative bacillary elements, without peripheral clubs.

It is easily possible to fulfil Koch's postulates by the rabbit inoculations. Pure cultures, as obtained from the lesions of the patient, caused the death of Rabbits 1 and 2 and produced in them lesions

TABLE III

Inoculation of Animals

Animal	Date	Material	Culture	Dosage	Method of inoculation	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
				cc.						
Rabbit 1	1/14/33	Liver, patient	24 hour pure brain broth	1.0	Intravenous	Severe general reaction	Died 1/15/33	1	Granulomatous foci liver, lungs, spleen	Liver and spleen
Rabbit 2	1/15/33	Liver, patient	48 hour pure brain broth	0.3	Intravenous	Slow general reaction	Died 1/30/33	15	Granulomatous foci liver, cecum, lungs, spleen, kidney	Spleen
Rabbit 3	1/20/33	Spleen, Rabbit 1	24 hour pure brain broth	0.8	Intravenous	Slow general reaction	Died 1/31/33	11	Granulomatous foci liver, lungs, spleen, kidney	Liver
Rabbit 4	1/25/33	Spleen, Rabbit 1	24 hour pure brain broth	1.5	Intravenous	Slow general reaction	Died 1/30/33	5	Granulomatous foci liver, lungs, spleen	Blood, liver, spleen
Rabbit 5	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, no general reaction	Killed 3/14/33	31	Granulomatous foci lung, subcutaneous abscess	Subcutaneous abscess
Rabbit 6	2/11/33	Spleen, patient	24 hour pure brain broth	2.5	Subcutaneous	Abscess site inoculation, general reaction, rapid terminal failure	Died 2/18/33	7	Granulomatous foci liver, spleen, abscess site inoculation	Blood, liver, spleen, subcutaneous abscess
Mouse 1	1/12/33	Blood patient, ante mortem	Original 24 hour brain broth	0.2	Subcutaneous	Slight local induration, no general reaction	Killed 2/9/33	28	Granulomatous foci spleen	Accidentally lost
Calf 1	2/1/33	Spleen, patient	24 hour pure brain broth	5.0	Subcutaneous	Mild general reaction, fever 2 days, bloody diarrhea, local swelling, recovered	Killed 3/29/33	57	No lesions	Negative

similar to those revealed at autopsy of the patient. Pure cultures of the organism as inoculated were obtained from the liver and spleen. Rabbits 3 and 4 were inoculated with the culture that had been isolated from Rabbit 1, and there also the lesions were unmistakably almost identical with those observed at autopsy of the patient. The organism was again isolated from the lesions of these rabbits in pure culture and proved to be identical with the original culture. In only one of the intravenously inoculated rabbits, Rabbit 4, was the blood culture positive at the time of death.

Subcutaneous inoculation of two rabbits proved fatal in one, the rabbit dying on the 7th day of septicemia. A local abscess was produced, as well as lesions in the liver and spleen. The other rabbit that received subcutaneous inoculation survived and was killed on the 31st day after inoculation. It revealed a well defined encapsulated local abscess and a few healing granulomatous abscesses in the lungs. In the lesions of Rabbit 6 colonization of the organisms in the disseminate lesions was a prominent feature. In Rabbit 5 organisms were not identified in the tissue sections. In both, however, cultures were positive.

Mice: Judging from the inoculations of one white mouse (Table III) mice are practically non-susceptible. Subcutaneous inoculation did not appear to provoke any illness or local lesion. The animal was killed on the 28th day; a few small, typical, chronic granulomatous foci were found in the spleen only.

Cattle: One calf was inoculated and results were essentially negative (Table III).

Guinea Pigs: These animals exhibited a variable susceptibility, although in all but one some lesions were produced. The results of subcutaneous inoculations are summarized in Table IV. Although local lesions were produced in all but Guinea pig 2 it will be noted that none of the animals died from the infection. Guinea pig 8 died on the 16th day after inoculation; pneumonia and not *Actinobacillus* infection was the cause of death. The pigs usually manifested slight disability, chiefly refusing food and losing weight for a few days, then generally recovered. The most severe reactions were encountered in Guinea pigs 1 and 4. Both were very ill when they were killed and probably would have died as a result of actinobacillosis. The severity of the illness of Guinea pig 1 was accounted for at autopsy by general peritonitis.

TABLE IV

Subcutaneous Inoculation of Guinea Pigs

Guinea pig	Date	Material	Material inoculated	Dosage	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/12/33	Spleen, patient	Triturated abscess spleen	Saline suspension 1 cc.	Abscess site inoculation, severe general reaction	Killed 1/18/33	6	Granulomatous foci liver, spleen, lungs, subcutaneous abscess, general peritonitis	Subcutaneous abscess, peritoneum, liver, spleen, blood
2	1/12/33	Lung, patient	Triturated abscess lung	Saline suspension 1 cc.	Small abscess site inoculation, mild general reaction, recovery	Killed 3/14/33	61	No lesions	Negative
3	1/12/33	Blood, patient, ante mortem	Original 24 hr. brain broth ante mortem culture	Brain broth culture 1 cc.	Small abscess site inoculation, drainage 50th day, no general reaction	Killed 3/14/33	61	Granulomatous foci liver, lungs, subcutaneous abscess	Subcutaneous abscess
4	1/12/33	Liver, patient	Triturated abscess liver	Saline suspension 1 cc.	Large abscess site inoculation, severe general reaction	Killed 2/4/33	23	Granulomatous foci liver, spleen, lung, subcutaneous abscess	Negative (contamination)
5	1/25/33	Blood, patient, ante mortem	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/9/33	15	Granulomatous foci liver, spleen, lungs, cutaneous ulcer	Negative (cultures lost)
6	1/25/33	Liver, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess and ulcer site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, lung, cutaneous ulcer	Negative
7	1/25/33	Spleen, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, mild general reaction	Killed 2/20/33	26	Granulomatous foci liver, cutaneous abscess	Subcutaneous abscess
8	1/25/33	Lung, patient	24 hour pure brain broth culture	Pure brain broth culture 2 cc.	Abscess site inoculation, severe general reaction	Died 2/10/33	16	Granulomatous foci liver, lymph node, spleen, cutaneous abscess, pneumonia	Subcutaneous abscess

Subcutaneous abscesses formed at the sites of inoculation in all of the animals except Guinea pig 2, in which lesions could not be found at autopsy. In two instances the abscesses began to drain after a few days and a large purulent ulcer with necrotic base and undermined borders formed. In Guinea pig 6 the ulcer began to heal about the 20th day and when the animal was killed, on the 26th day, the ulcer was almost healed. In Guinea pig 5, however, the ulcer remained about 2 cm. in diameter and appeared to be progressing at the time the animal was killed. In Guinea pig 3 a small abscess developed which persisted, and on the 50th day a small sinus appeared, through which purulent material drained. In the eight guinea pigs inoculated subcutaneously small, discrete, grayish white, necrotic foci were found in the livers of seven, in the lungs of five, and in the spleens of four. One of the guinea pigs had general peritonitis of serofibrinopurulent type and one had an enlarged inguinal lymph node containing necrotic foci.

Microscopically the lesions in these animals are similar to those in the rabbits. The local subcutaneous abscesses are composed centrally of a large area of necrotic exudate in which a few polymorphonuclear leukocytes are preserved, but usually only nuclear detritus and eosin-staining granular material are present. Surrounding this is a wide zone of well preserved polymorphonuclear leukocytes that blends peripherally with a zone of granulation tissue in which fibroblasts and young blood vessels form the supportive structure. Intermingled with these cells are polymorphonuclear leukocytes, large mononuclear phagocytic cells, plasma cells, lymphocytes and other cells, sometimes quite numerous, interpreted as endothelioid. In the outer portions bacterial aggregations exist in colony formation; these are Gram-negative and not associated with peripheral clubs. In the viscera the lesions tend toward chronic abscesses, with central collections of polymorphonuclear leukocytes and peripheral granulomatous reaction. In some of the lesions necrosis is a prominent feature, but in others it is slight. In the older lesions the peripheral zone has become distinctly fibrous, and in others this fibrosis has progressed to involve most of the focus, as though to form a cicatrix of a healed lesion. However, in the more severe general infections, as in Guinea pigs 1 and 4, all stages of lesions exist from focal areas of necrosis to abscesses, as described. Intermediate lesions frequently resemble endothelioid tubercles, or wide zones of

TABLE V

Intraperitoneal Inoculation of Guinea Pigs

Guinea pig	Date	Culture	Culture dosage 1 cc.	Results	Date of death	Days after inoculation	Autopsy data	Cultures recovered from
1	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, conjunctivitis	Killed 1/18/33	4	Fibrinopurulent peritonitis, granulomatous foci in peritoneum, liver, spleen, lungs, lymph nodes	Liver, peritoneum, spleen
2	1/14/33	Spleen, patient	24 hour pure brain broth	Severe general reaction, conjunctivitis	Died 1/20/33	6	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs, kidneys, lymph nodes	Spleen
3	1/25/33	Blood, patient	24 hour pure brain broth	Severe general reaction	Died 2/3/33	9	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs	Liver, peritoneum
4	1/25/33	Liver, patient	24 hour pure brain broth	Severe general reaction	Died 1/31/33	6	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen, lungs, adrenals, lymph nodes	Blood, liver, peritoneum
5	1/25/33	Spleen, patient	24 hour pure brain broth	Severe general reaction	Died 1/30/33	5	Fibrinopurulent peritonitis, granulomatous foci in liver, spleen	Blood, liver, peritoneum
6	1/25/33	Lung, patient	24 hour pure brain broth	Mild general reaction, recovery, subcutaneous abscess with draining sinus	Killed 2/20/33	26	Subcutaneous abscess with draining sinus, granulomatous foci in liver, lungs, adrenals, local peritonitis, periorchitis	Negative

central necrosis with a slight exudative and granulomatous proliferative reaction peripherally. In most instances colonies of bacteria are associated with the lesions; in the early lesions in the center of the necrotic focus, and in older lesions toward the border of the zone of necrosis or in the area of exudation. These are frequently found as large compact masses not retaining the Gram stain, and sometimes with just a faint suggestion of peripheral clubs.

Following intraperitoneal inoculation of guinea pigs with pure cultures (Table V), the animals became ill in from 24 to 48 hours; they refused food, were weak and listless, the hair became roughened, they lost weight and the abdomen became tender. All but two of the animals, Guinea pigs 1 and 6, died in from 5 to 9 days. Guinea pig 1 was moribund at the time it was killed and would have died within a few hours. Guinea pig 6, however, after an initial slight general reaction, apparently recovered with only a local abscess and draining sinus at the site of inoculation. It was killed on the 26th day. In none was the Strauss reaction elicited clinically. At autopsy all the animals were found to have peritonitis. In only one, Guinea pig 6, was the reaction local; it was confined to an area beneath the parietal peritoneum of the ventral wall, and adherent loops of small intestine and omentum. A sinus tract through the peritoneum and abdominal muscles formed a subcutaneous abscess and a draining sinus through the skin. The peritonitis was of a peculiar type. The peritoneal cavity contained a few cc. of clear amber fluid. The surface of the liver and spleen and usually the omentum and stomach were covered by a thick, adherent, grayish yellow, fibrinopurulent exudate. These structures were frequently adherent to one another. Over the coils of the small intestines there were frequently isolated or discrete, small, grayish yellow foci that resembled tubercles. In two guinea pigs (Guinea pigs 1 and 6) the same appearance was presented on the tunica of the testis and epididymis. The livers of all the animals were involved by fine grayish yellow points of apparent necrosis. Similar lesions, although sometimes more distinct, were observed in the lungs and spleen of five animals. In two animals the suprarenal glands were slightly involved. In one animal the mediastinal lymph nodes were involved and in one, Guinea pig 6, the inguinal nodes. In two animals, as has been noted, periorchitis existed.

Microscopically, except in Guinea pig 6, the lesions are usually relatively acute, but throughout the various involved organs and the

various animals, lesions of varying age are presented. These are observed in their simplest form as areas of necrosis at the point of lodgment of bacterial masses in the capillaries, to focal abscesses or focal granulomatous, tubercle-like structures. The early lesions consist of a central compact aggregation of bacteria surrounded by a zone of necrosis in which a few polymorphonuclear leukocytes are seen. Slightly older lesions reveal a peripheral zone of endothelioid cells, with perhaps a greater number of polymorphonuclear leukocytes collected around the bacterial colony (Fig. 5). In other instances lesions of this stage reveal only central necrosis with a rim of polymorphonuclear leukocytes and endothelioid cells, or with the central zone composed entirely of polymorphonuclear leukocytes without necrosis. Central or eccentric masses of bacteria are sometimes observed in the latter lesions, although they not infrequently are entirely absent. Still older lesions are in certain instances predominatingly endothelioid tubercles, sometimes with central polymorphonuclear neutrophilic exudation and at other times with almost solid endothelioid cells. In Guinea pig 6 the lesions are more chronic (26 days). They consist of central polymorphonuclear leukocytes with considerable necrotic cellular débris, a peripheral rim of endothelioid cells and fibroblasts or fibrous connective tissue. These lesions are in the liver, suprarenal glands, lungs, tunica of the testes and subcutaneous tissue. In these chronic lesions the masses of bacteria are not visible.

The Pathological Changes in Bovine Actinobacillosis

The lesions of bovine actinobacillosis are grossly like those of actinomycosis, but although true bovine actinomycosis usually involves the jaw bones, where it produces rarefying osteitis, bovine actinobacillosis usually affects the soft tissues of the head and neck. Specifically the parts most often affected are the tongue, palate, pharynx, cheeks, skin of face and lymph nodes of the submaxillary and cervical regions. The lungs have been described also as the site of affection.

Grossly the smaller bovine lesions may be described as appearing like areas of soft granulation tissue with central suppuration, the central zone of exudation gradually enlarging and the peripheral zone of granulation tissue gradually developing a thick fibrous capsule. The pus is thick, viscid or gelatinous and greenish yellow. In it the sulphur granules are found.

The microscopic characteristics of the lesions, as determined in bovine material from which Thompson isolated the *Actinobacillus* in pure culture, will be appended for comparative study. These corresponded with the previously published descriptions of such lesions. They also were essentially the same as those that were found in our patient, and those that were produced in our experimental animals. The central portion of the lesion, which was essentially a granulomatous abscess, was composed of exudate in which fairly well preserved polymorphonuclear leukocytes predominated. Small granules, the colonies of bacteria with peripheral clubs, were fairly abundant in the exudate that adhered to the wall of the abscess. This wall consisted of granulation tissue of loose texture, the principal supportive structure of which consisted of fibroblasts and newly formed blood vessels, throughout which were interspersed lymphocytes, large phagocytic mononuclear leukocytes, plasma and endothelioid cells. Polymorphonuclear leukocytes gradually diminished in number as the peripheral portions were reached, except for focal aggregations. The uniform structure of the wall of the abscess was somewhat altered by the presence of small abscesses, similar, although much smaller, to the central large one. This gave the entire wall more or less of an alveolar appearance. The smaller abscesses were composed of a central portion in which one to several compact bacterial aggregations with peripheral clubs existed. When these were stained by Gram's method only Gram-negative bacillary forms in compact aggregations with peripheral clubs were revealed. They were entirely devoid of Gram-positive branched filaments. Surrounding the bacterial colonies there were well preserved polymorphonuclear leukocytes, some necrosis and occasionally giant cells. Peripherally there was an encircling zone of slightly more compact granulation tissue than was found elsewhere in the wall of the large abscess, with a tendency to strands of fibrous connective tissue as a final encapsulation for the alveolar structure. Surrounding the entire lesion was a dense encapsulation of fibrous tissue.

Epidemiology

The patient's immediate family was carefully questioned concerning possible sources of infection, but without eliciting information of value. It was stated most emphatically that he had had no contacts with cattle or other animals, that he had never drunk milk as a

beverage and that all milk used at his home was pasteurized dairy milk. He had always insisted on his meats being well cooked. There had been no local injuries or lesions about the hands or other exposed surfaces of the body.

The method of natural spread of the disease among cattle is not known, but it is presumed that the organism is strictly parasitic and contact with infected animals or carriers constitutes the mode of dissemination. The disease usually occurs sporadically in cattle and is not highly contagious, although Lignières and Spitz report its occurrence in cattle in epizoötic form.

Summary

Three cases of actinobacillosis in man are now well authenticated. The case reported here offers the first opportunity for pathological study of the lesion in a human being. The lesions are essentially similar to those that have been described in cattle, except that in man the lesions are much more widespread, and sulphur granules, such as occur in bovine lesions, apparently do not occur. In the present case the lesions may be described as granulomatous abscesses severely affecting the lungs, liver and spleen. Lesions similar to those observed in man and cattle were produced in experimental animals. Glanders and tularemia were considered in the differential diagnosis, but both seemed to be definitely ruled out by the bacteriological and serological investigations. The organism isolated from our case reveals a close cultural and antigenic relation to *Actinobacillus lignièresi*, and a more distant relation to *Pfeifferella mallei* and *Bacillus whitmori*. Thompson has previously shown that *Actinobacillus lignièresi* of bovine and human origin, *Pfeifferella mallei* and *Bacillus whitmori* are antigenetically interrelated, and has proposed that they be included in a common genus. This relationship, as far as the organism in our case is concerned, is also confirmed from the pathological viewpoint, for the lesions in man and in experimental animals were similar to glanders, as well as exhibiting similarities to bovine actinobacillosis. Although the organism under consideration revealed minor cultural differences and somewhat different antigenetic phenomena from a typical strain of *Actinobacillus lignièresi* of bovine origin, nevertheless, it seems justifiable to regard the organism in this case as a variant of the usual bovine strain of *Actinobacillus lignièresi*.

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DESCRIPTION OF PLATE

Plate 101

Fig. 1. Spleen of patient revealing multiple granulomatous abscesses.

Fig. 2. Granulomatous abscess from lung of patient. In the center of the abscess polymorphonuclear neutrophilic leukocytes predominate with a few mononuclear phagocytes and lymphocytes. Peripherally endothelioid cells and fibroblasts are seen just within and intermingling with a granulation tissue encapsulation. Hematoxylin and eosin stain. × 160.

Fig. 3. Portion of large granulomatous abscess from liver of patient. Central necrosis, zone of leukocytes and early peripheral endothelioid cell proliferation may be seen. Large masses of bacteria in colony formation, more deeply stained, may also be seen. Hematoxylin and eosin stain. × 45.

Fig. 4. Spleen of Rabbit 3 inoculated intravenously with pure culture of *Actinobacillus*, as isolated from the liver of the patient after passage through Rabbit 1. The rabbit died the 11th day after inoculation. The resemblance to the lesions in the patient's spleen may be noted (Fig. 1).

Fig. 5. Liver of guinea pig inoculated intraperitoneally. Two well defined lesions are revealed. Colonies of *Actinobacillus* may be noted in the centers of the lesions. Surrounding the bacterial colonies are polymorphonuclear neutrophilic leukocytes and necrosis, and at the periphery is a well defined zone of endothelioid cell proliferation. Hematoxylin and eosin stain. × 110.

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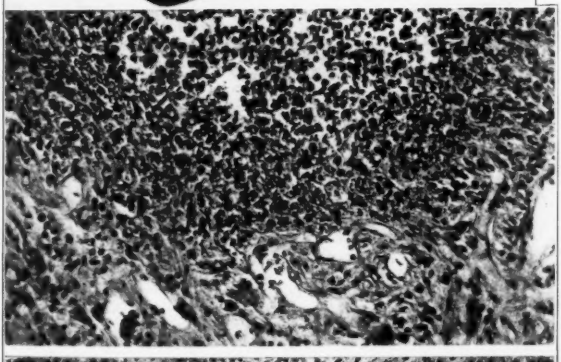

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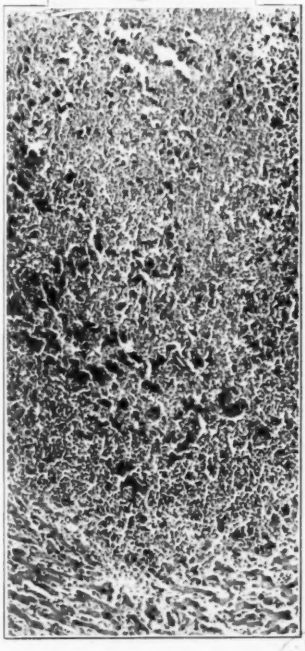

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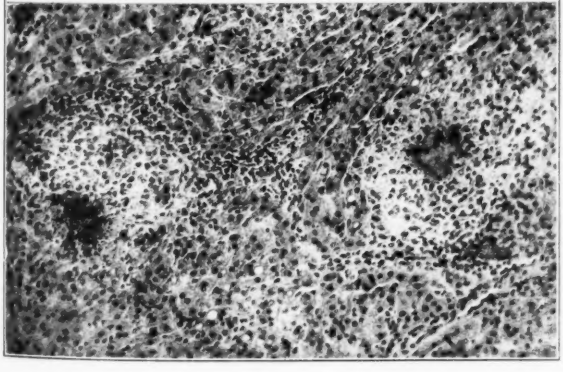

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Beaver and Thompson

Actinobacillosis of Man

ERYTHROLEUCOBLASTOSIS IN THE NEWBORN *

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Numerous cases of congenital hydrops have been described since 1878, but Schridde [1] in 1910 directed attention to the changes in the blood-forming tissue in congenital hydrops, accompanied by a high degree of anemia. Since then it has often been called Schridde's type of congenital hydrops, in contradistinction to general edema. His findings have been confirmed time and again by numerous investigators, who also have described a reparative growth of erythroblastic tissue and a general edema, which had no etiological relation to congenital syphilis or to a coexistent maternal nephritis.

After careful investigation of a case Rautmann [2] in 1912 expressed the opinion that the real condition was a primary erythroblastic process and therefore coined the term "erythroblastosis."

Thirty-four cases from the German literature were reviewed by Hartmann [3] in 1928. He noted the frequent coincidence of maternal renal disturbances, but later concluded that the fetal condition was primary and the maternal renal disease secondary, due to a toxicosis produced by the fetus. Oberndorfer [4] found congenital hydrops in one of twins and concluded, therefore, that a maternal etiological factor was out of the question.

In 1921 von Gierke [5] found the pathological changes described by Schridde in a non-hydropic baby with "Kernikterus," and he established the hypothesis that these diseases might be related and that they might represent different complexes of symptoms, but be due to the same cause. The observation of the coincidence of icterus gravis neonatorum, which can occur with or without "Kernikterus," with fetal erythroblastosis has been so abundantly confirmed that the occurrence is undoubted. He believed the condition not to be a primary destructive anemia.

Kleinschmidt [6] found a high degree of erythroblastosis in icterus neonatorum, the blood picture corresponding to that of an embryo between three and five months. Volhard [7] believed there existed a

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similarity between cases of congenital edema and icterus gravis neonatorum and that there was a pronounced familial disposition, as frequently several offspring of one mother showed one or the other condition.

Von Gierke in 1930 [8] examined a newborn girl baby who died one hour after birth. This child was a sister of the child with "Kernikterus" described by him in 1921. The liver and spleen were greatly enlarged, with a tear in the capsule from which there was some bleeding. Blood smears showed numerous normoblasts, megaloblasts and a large number of myelocytes and myeloblasts. The spleen, liver and bone marrow showed a high degree of erythroblastic and myeloblastic growth, with smaller masses of these cells in the kidney, skin and heart muscle. This form von Gierke called leucoblastosis, having a relation on the one hand to erythroblastosis and on the other to a few cases of fetal leucemia, which had been described. He concluded that there is a disturbance of fetal hematopoiesis in which either the erythroblastic or the leucoblastic apparatus, or both, are concerned. Accordingly he divided fetal blood diseases into:

1. Fetal erythroblastosis
2. Fetal erythroleucoblastosis
3. Fetal leucoblastosis

The first and second were frequently accompanied by congenital hydrops or by icterus gravis neonatorum. The third was like extrauterine leucemia. The etiology of this condition was not known.

Furthermore, von Gierke concluded that there was a tendency for several cases to occur in the same family, or cases of both congenital hydrops and icterus neonatorum. In the majority of cases the mother was perfectly well so that maternal illness was only an incidental occurrence. He believed that these conditions were each a part of one disease entity, which under changes in circumstances involved different components of the hematopoietic system. Microscopically these conditions presented a marked increase in immature forms of the erythroblastic and leucoblastic series in the blood and also in the organs, particularly in the liver and spleen. The erythroblastic disease was frequently found with an accompanying congenital edema or an icterus gravis neonatorum. Also, in congenital hydrops there was frequently a slight icteric tint in the tissues. It seemed possible that both manifestations were due to the same fetal

blood disease; congenital hydrops appeared early, was intrauterine, causing a stillbirth or an infant living only a few hours, while icterus gravis neonatorum usually appeared in the first few hours after birth. With congenital hydrops and icterus gravis neonatorum there was frequently a hyperplastic placenta, but the reason for this was not known.

Von Gierke also reported a case of fetal erythroleucoblastosis in which the liver and spleen showed a marked increase in erythroblastic and leucoblastic myelogenous tissue in the hematopoietic areas. The kidneys, heart muscle, skin and gastric mucosa also showed extravascular myeloid nodules. A large part of the leucoblasts gave the oxydase reaction. The picture closely resembled that of myelogenous leucemia, except for the marked increase in erythroblasts in the blood and in the cellular infiltrations in the tissues.

In 1930 Hueper and Mullen [9] described two cases of erythroblastosis with congenital hydrops and believed these findings were the effects of the toxic action of an unknown substance in the circulation upon the blood and the vascular walls, resulting in the persistence of the hematopoietic system at the high developmental level of the seventh month, or even at a higher level, as an attempted reparative or compensatory hyperplasia of this tissue. Iron pigment was found in the liver and spleen, and bile casts in the bile ducts of the liver, indicating the existence of a marked degree of blood destruction.

Six cases of erythroblastosis were reported by Ferguson [10] in 1931, three of them showing jaundice, two with a generalized edema and one with neither jaundice nor edema. All six showed marked enlargement of the liver and spleen, and active hematopoiesis in bone marrow, liver, spleen and other organs. In two of his cases there was bile stasis in the liver cells and bile capillaries.

In 312 autopsies on the newborn Salomonsen [11] in 1931 found five cases of congenital hydrops with fetal erythroleucoblastosis. There was marked hemosiderosis in the liver but none in the spleen. One of his cases gave opportunity for examination of blood smears during life. The hemoglobin reading was 81 per cent and the red blood cell count was 3,765,000 with 123,700 nucleated cells, of which 49 per cent were nucleated red blood cells. All forms of immature blood cells were present. The livers and spleens of fifty-two normal newborn infants were examined histologically for iron pigment, but the

hemosiderin found in congenital hydrops did not differ enough from that occurring normally to draw any conclusions in regard to a primary destructive anemia from this one finding. Salomonsen believed that a familial relation existed between congenital hydrops and congenital icterus gravis. He concluded that it was correct to ascribe fetal erythroleucoblastosis to a primary constitutional disturbance of the "Anlage" of the hematopoietic system, being a special fetal disease group; and that this group included congenital hydrops and icterus gravis congenitas as well.

As evidence for the familial occurrence of this disease complex Plaut and Bullard [12] in 1926 reported a family in which two infants died of icterus gravis and another had typical congenital hydrops with erythroblastosis. They believed that autopsy examinations of babies with icterus gravis familiaris and of non-hydropic babies of mothers who had previously given birth to hydropic infants, would reveal more instances of "Kernikterus" and give more evidence of a connection between fetal hydrops, erythroblastosis, icterus gravis familiaris, "Kernikterus" and other forms of jaundice in the first days and weeks of life. The origin of this disease complex was not known but the child affected was usually immature, the thymus small and there was no maternal, paternal or other condition to give any clue.

Clifford and Hertig [13] in 1932 suggested that a subsequent pregnancy might produce a different clinical type of disease. It seemed likely that it might result as a defect of the germ plasm or of the fertilized ovum. Erythroblastosis appeared to run the major portion of its course *in utero* and in the first days of life. It occurred in either sex and in any race, but the first born was usually spared. Congenital hydrops appeared relatively frequently, 1 to 1200 cases at the Boston Lying-In Hospital. Icterus gravis was considered relatively infrequent while, in the same hospital, there occurred seven proved cases of erythroblastosis during a period of nine months, or 1 in 340 births. These observers found the placental villi enlarged, edematous and somewhat hyperplastic, and also noted that some of these babies were covered with a thick, golden yellow, vernix caseosa, although this occurred also on a few apparently normal babies.

Report of Case

Clinical History: C., a baby girl, was born in the University Hospital at 10:21 P.M., Jan. 7, 1931. The mother, aged 17 years, had always been in good health. Her father, aged 53 years, had diabetes mellitus and her mother, aged 48 years, was well. She had one brother and one sister living and well. There was no history of any constitutional disease in the family. Nothing was learned regarding the baby's paternal ancestry as the mother was unmarried.

The mother's health had been good throughout pregnancy. The cervix was fully dilated 11 hours after the onset of labor. The head was born easily, but the body of the child was delivered with considerable difficulty because of a markedly distended abdomen. Following the birth of the child 2000 cc. of clear, straw-colored amniotic fluid were discharged from the uterus. The onset of respiration was delayed and it was necessary to resort to artificial respiration.

Examination of the child, following delivery, revealed a female infant weighing 2040 gm., total length 41 cm., vertex to rump 25 cm., biparietal diameter 7.5 cm., suboccipital-bregmatic 8 cm., occipital-mental 11 cm., and occipital-frontal diameter 10 cm. The circumferences were as follows: suboccipital-bregmatic 27 cm., occipital-frontal 29 cm., and bis-acromial 25 cm. The abdomen was markedly distended by fluid, was soft, the percussion note was dull and a definite fluid wave could be obtained. The greatest circumference of the abdomen was 35 cm.

The child had several attacks of cyanosis shortly after birth and it was necessary to resort to oxygen with 5 per cent carbon dioxide to stimulate respirations. At 4:45 A.M., Jan. 8, 1931, about 6 hours after birth, respirations ceased. Permission for autopsy was granted and this was performed at 11:00 A.M.

Laboratory Findings: Routine diagnostic blood Kahn test on the mother gave negative results, as did the Kahn test on blood from the umbilical cord. The latter specimen contained bile.

Autopsy Report

The external examination of the body showed nothing additional to the findings given in the clinical history. There was marked cyanosis of the head, face and neck, and also numerous brownish red spots in the skin over the abdomen. The scalp showed marked edema over the left parieto-occipital region, with areas of ecchymosis. The very large liver could be palpated through the thin abdominal wall.

Around the brain stem and over the inferior portion of the cerebellum there was a massive and relatively recent blood clot that entirely filled the available space beneath the tentorium and was subarachnoid in distribution. This hemorrhage apparently arose from numerous small pial veins. The cerebrum showed marked congestion and edema. The cerebellum showed diffuse subarachnoid hemorrhage. There was marked congestion and edema of the cerebellar substance.

The panniculus was scant and was of a very faint yellowish color, somewhat suggestive of icterus.

The peritoneal cavity contained 500 cc. of clear, golden yellow fluid without fibrin masses.

The cardiac apex was at the sixth intercostal space in the anterior axillary line. The heart was 50 per cent larger than the cadaver's right fist and lay nearly horizontally. The anterior aspect was composed of the right ventricle, which showed extreme hypertrophy and measured 7 mm. in thickness. The apex was composed entirely of the right ventricle. The left ventricular wall was 5 mm. thick. The muscle was pale red and showed no gross lesions. The mitral valve was normal in size. The flaps were negative. The aortic valve was normal in size. There was no stenosis of the first portion of the aorta and the cusps were normal. The tricuspid valve showed very marked dilatation, admitting the tip of the first finger. It appeared incompetent. The pulmonary valve showed moderate dilatation but the cusps showed normal development. The pulmonary artery was dilated to approximately twice the usual size. The ductus arteriosus was larger than the arch of the aorta and was approximately three times the size of the normal ductus. There was a redundant semilunar fold guarding the foramen ovale.

The spleen was approximately twice the normal size. The capsule was somewhat thickened. The parenchyma was dark red and no lymphoid structure could be seen. The stroma appeared to be relatively increased.

The liver was moderately enlarged, dark red in color and the capsule was smooth. On section minute pale areas 0.5 mm. in diameter were observed, but the exact location of these pale areas with reference to the lobule could not be determined. The ductus venosus was dissected and found to be patent. The portal vein was investigated outside of the liver and no thrombosis found.

The kidneys appeared normal in size and showed well marked congestion. The ureters were moderately dilated, the right more than the left.

Microscopic Examination

Histological sections were prepared with hemalum and eosin; erythrosin and hemalum; erythrosin, picric acid and hemalum; Weigert's elastica stains; and silver stains by the coverslip method.

Spinal Cord: Marked congestion of meninges and cord substance is present.

Brain and Meninges: There is marked congestion and edema of meninges and congestion of brain substance. Small petechial hemorrhages are found beneath the ependyma of the ventricles. There are increased wandering cells in the meninges. Small calcified thrombi occur in the brain substance. Extensive intrameningeal hemorrhage is present.

Heart: Shows congestion. The muscle appears well developed for the age of the subject.

Aorta: Negative.

Lungs: The lungs show acute passive congestion. Aspirated meconium and epithelial cells from the amniotic fluid are found. The peribronchial nodes show lymphoid hyperplasia. Small hemorrhages are present. The fat stain shows no fat emboli.

Tongue: Negative.

Pharynx: The lymphoid tissue is hyperplastic. There are multiple perivascular foci of active hematopoiesis.

Larynx: Acute passive congestion is present.

Esophagus: Hematopoietic foci are seen in the esophageal wall.

Thyroid: The colloid is more abundant than usual at birth.

Thymus: The medulla is hypoplastic with very small corpuscles of Hassall. Areas of active hematopoiesis occur around blood vessels in the medulla. Congestion is present.

Spleen: An intense exacerbation of chronic passive congestion is seen. The stroma is increased. There are small foci, chiefly perivascular, of active hematopoiesis. Special stains show no hemosiderin.

Diaphragm: The muscle appears well developed. There are small perivascular collections of hematopoietic tissue.

Umbilical Cord Stump: Fresh thrombi without organization are seen.

Appendix: Marked mucin formation and passive congestion is present.

Small Intestine: There are small areas of hematopoiesis in the subserosa. There are lymphoid hyperplasia, marked passive congestion and also edema of the subserosa.

Duodenum: Marked chronic passive congestion is present.

Stomach: The stomach shows marked passive congestion and increased mucin formation.

Pancreas: Diffuse hematopoiesis is present throughout the pancreas. There is congestion and some increase in young connective tissue. The portal vein radicles are greatly distended and their walls thickened. The arteries show hypertrophy of the walls with marked development of the internal elastic lamina.

Liver: The prominence of the islands of Glisson is found to be due to infiltration with lymphocytes and cells of types found in bone marrow, an active and extensive hematopoiesis. There are also many myeloid giant cells present and many nucleated forms with eosinophilic cytoplasm. In the peripheral portion of the lobule many liver cells contain a yellow-brown pigment. By special staining it is found that a large part of this pigment is hemosiderin, the remainder being largely formol-precipitated hemoglobin. Small collections of bile pigment are present but there is no evidence of an obstructive jaundice. In some of the islands of Glisson the epithelium of the bile ducts shows degenerative changes and has been in part replaced by large spherical cells with large, deeply staining nuclei. These cells vary slightly in size, and in some the cytoplasm appears finely granular. Fat stains show a lipoidosis in the reticuloendothelial cells.

Adrenals: Marked hypoplasia of the medulla and congestion of the cortex is seen. In the medulla are small vessels showing complete calcification of old thrombi.

Kidneys: Marked congestion is present and there is perivascular hematopoiesis, even in the cortex. There are large cells in the renal tubules, apparently the result of attempted repair of the damaged renal epithelium. These cells resemble so closely the large cells found in the bile ducts that it appears evident they must have had a similar origin.

Bone Marrow: The bone marrow is markedly cellular with active hematopoiesis throughout, the prevailing cells being of the red blood cell series.

Urinary Bladder: Negative.

Vagina and Rectovaginal Septum: There is hematopoietic tissue around most of the smaller blood vessels in the submucosa and fascia.

Internal Genitalia: Negative, except for congestion.

Lymph Nodes: The lymph nodes are hyperplastic, with a diffuse distribution of hematopoietic tissue throughout. The follicles are reduced in size and are represented by small aggregates of lympho-

blasts and lymphocytes, suggesting splenic corpuscles in their relation to arterioles.

Placenta: The villi of the placenta are somewhat plumper than normal. This is due more to edema than to increased stroma. Particularly in the larger stems, however, there is an increased number of cells that tend to be arranged in small aggregates about blood vessels. In these foci numerous cells with eosinophilic cytoplasm and numerous cells of the myelocyte series occur. There are no evidences of a syphilitic chorionitis and staining for spirochetes gives negative results.

Blood: (No clinical blood studies were made.) The blood as seen in the blood vessels of the tissues shows a very high proportion of nucleated cells in the red blood cell series.

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There seems no doubt that a definite familial tendency exists, since there have been enough reports of either erythroblastosis, congenital hydrops or icterus gravis in several children of the same mother to exclude mere coincidence. The familial occurrence quite properly brings up the question of whether or not the presence of congenital hydrops or erythroblastosis in one or more offspring is sufficient reason for advising against having additional children. Perhaps we are not prepared to answer this in the present state of our knowledge, as it is entirely possible that the lesser degrees of the condition may be treated successfully by transfusions, when recognized early. Reports of such successful therapy have already been made.

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3. In the lesser bile ducts and in some renal tubules numerous giant cells were found, apparently resulting from alterative changes in damaged epithelium.

4. The generic relationship and probable intrinsic constitutional etiology of congenital hydrops, icterus gravis and fetal erythroleucoblastosis of the newborn are pointed out and suggestions made as to future investigations along these lines.

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Pancreas: Diffuse hematopoiesis is present throughout the pancreas. There is congestion and some increase in young connective tissue. The portal vein radicles are greatly distended and their walls thickened. The arteries show hypertrophy of the walls with marked development of the internal elastic lamina.

Liver: The prominence of the islands of Glisson is found to be due to infiltration with lymphocytes and cells of types found in bone marrow, an active and extensive hematopoiesis. There are also many myeloid giant cells present and many nucleated forms with eosinophilic cytoplasm. In the peripheral portion of the lobule many liver cells contain a yellow-brown pigment. By special staining it is found that a large part of this pigment is hemosiderin, the remainder being largely formol-precipitated hemoglobin. Small collections of bile pigment are present but there is no evidence of an obstructive jaundice. In some of the islands of Glisson the epithelium of the bile ducts shows degenerative changes and has been in part replaced by large spherical cells with large, deeply staining nuclei. These cells vary slightly in size, and in some the cytoplasm appears finely granular. Fat stains show a lipoidosis in the reticuloendothelial cells.

Adrenals: Marked hypoplasia of the medulla and congestion of the cortex is seen. In the medulla are small vessels showing complete calcification of old thrombi.

Kidneys: Marked congestion is present and there is perivascular hematopoiesis, even in the cortex. There are large cells in the renal tubules, apparently the result of attempted repair of the damaged renal epithelium. These cells resemble so closely the large cells found in the bile ducts that it appears evident they must have had a similar origin.

Bone Marrow: The bone marrow is markedly cellular with active hematopoiesis throughout, the prevailing cells being of the red blood cell series.

Urinary Bladder: Negative.

Vagina and Rectovaginal Septum: There is hematopoietic tissue around most of the smaller blood vessels in the submucosa and fascia.

Internal Genitalia: Negative, except for congestion.

Lymph Nodes: The lymph nodes are hyperplastic, with a diffuse distribution of hematopoietic tissue throughout. The follicles are reduced in size and are represented by small aggregates of lympho-

blasts and lymphocytes, suggesting splenic corpuscles in their relation to arterioles.

Placenta: The villi of the placenta are somewhat plumper than normal. This is due more to edema than to increased stroma. Particularly in the larger stems, however, there is an increased number of cells that tend to be arranged in small aggregates about blood vessels. In these foci numerous cells with eosinophilic cytoplasm and numerous cells of the myelocyte series occur. There are no evidences of a syphilitic chorionitis and staining for spirochetes gives negative results.

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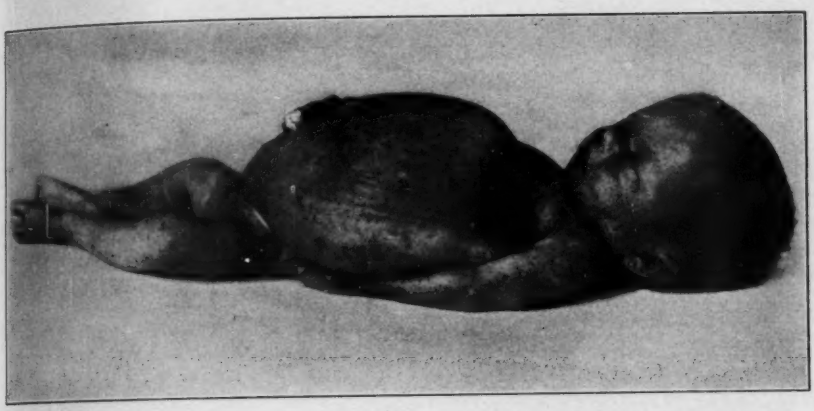
DESCRIPTION OF PLATES

PLATE 102

FIG. 1. Erythroleucoblastosis. Death at age of 6 hours. Extreme ascites. Multiple petechial hemorrhages and areas of pigmentation over abdomen and thighs.

FIG. 2. Marked hematopoiesis in liver in case of erythroleucoblastosis. Follicular arrangement about small bile duct. Hemalum and eosin stain. × 220.



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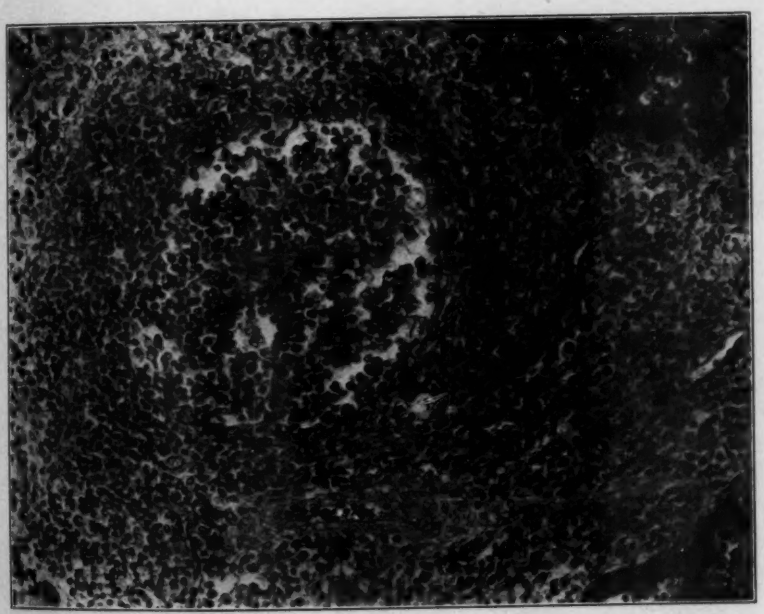

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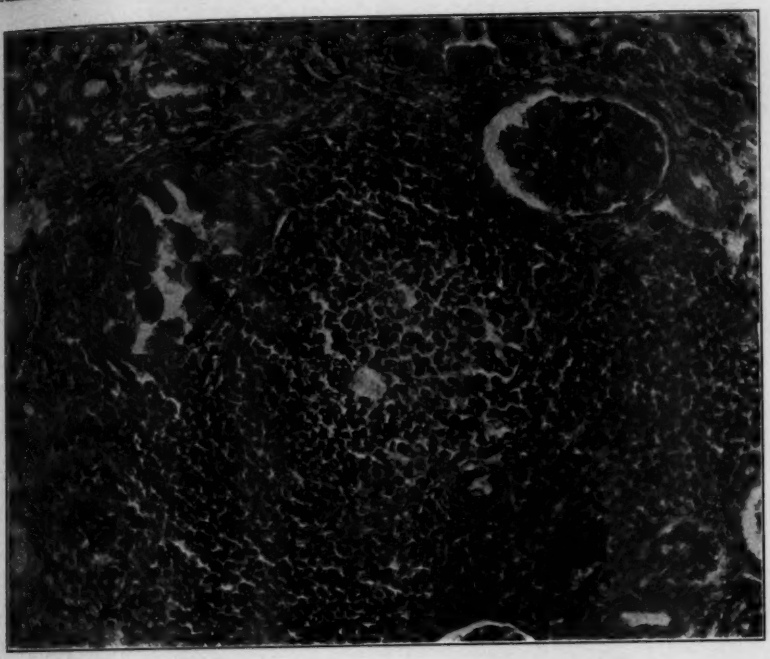
PLATE 103

FIG. 3. Marked hematopoiesis in kidney in same case as Figs. 1 and 2. Follicular arrangement of the leucoblastic tissue. These infiltrations are most marked in the deepest portion of the cortex and in the adjacent medulla. Hemalum and eosin stain. × 220.

FIG. 4. Small bile duct surrounded by hematoblastic tissue and showing in its lumen several mononuclear giant cells, probably derived from the epithelium. Hemalum and eosin stain. × 510.



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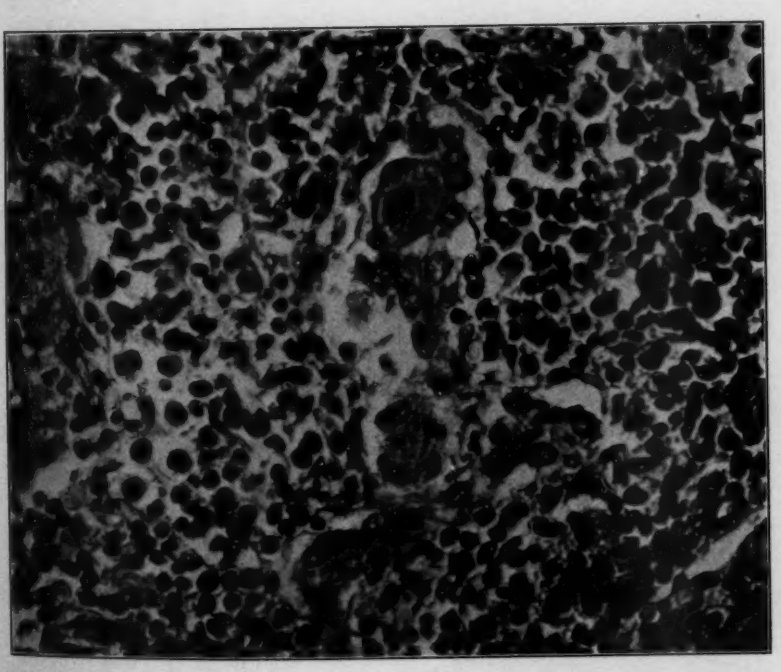

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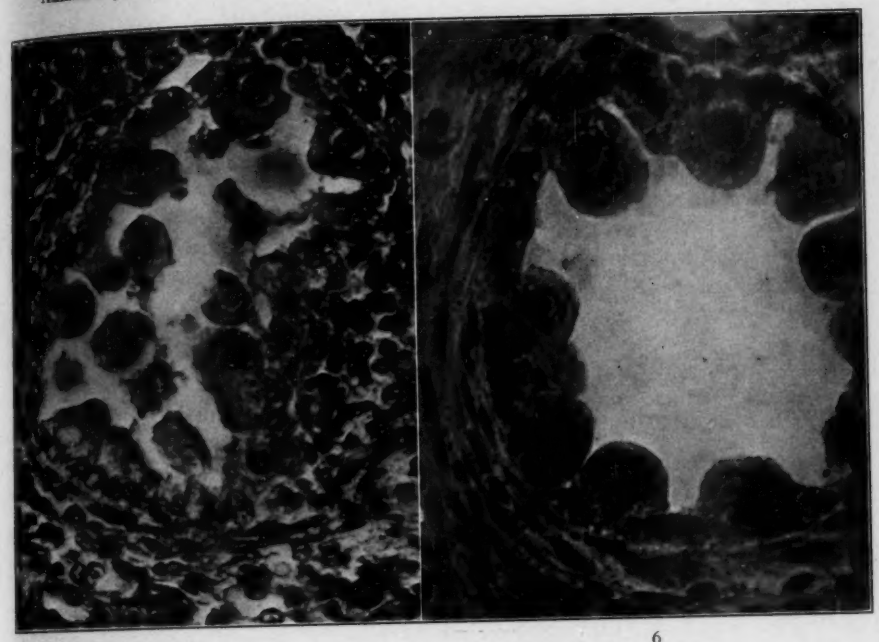
Wanstrom Erythroleucoblastosis in Newborn

PLATE 104

FIG. 5. Renal tubule embedded in hematoblastic tissue. This tubule shows numerous mononuclear giant cells, which in size, cytological detail and relation to the epithelium appear identical with those in the liver, as illustrated in the preceding figure. Hemalum and eosin stain. × 510.

FIG. 6. Large mononuclear cells in a renal tubule showing the small basic-staining granule lying beside the nucleus, as well as the numerous lipoid droplets within the cytoplasm. Hemalum and Sudan III stain. × 510.

FIG. 7. Hematopoietic tissue in stems of the central chorionic villi. Hemalum and eosin stain. × 220.



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7

Wanstrom Erythroleucoblastosis in Newborn

CONGENITAL HEART DISEASE WITH PULMONARY ARTERITIS*

INTERVENTRICULAR SEPTAL DEFECT, DEXTROPOSITION OF THE AORTA AND DILATATION OF THE PULMONARY ARTERY

HAROLD L. STEWART, M.D., AND BAXTER L. CRAWFORD, M.D.

(From the Pathological Department of the Jefferson Medical College and Hospital, Philadelphia, Pa.)

A solitary opening at the base of the interventricular septum in association with other defects ranks as the most frequent cardiac anomaly, the commonest of these combinations being the "Tetralogy of Fallot." A somewhat similar, though much rarer, combination consists of a ventricular septal defect, dextroposition of the aorta, and dilatation of the pulmonary artery instead of the usual stenosis. The first example of this condition was reported by Dalrymple in 1847; in 1897 Eisenmenger [1] presented the first complete study of such a case in which a correct ante mortem diagnosis had been made by his colleague Von Schrotter; and in 1927 Abbott [2] listed 8 cases, 3 of which she had studied personally. Clinically the condition can be differentiated from the tetralogy by the absence of or only a moderate degree of clubbing and cyanosis, the localization over the precordium of a harsh systolic murmur at the defect, not transmitted into the vessels, the occasional presence of a diastolic murmur of pulmonary insufficiency and the distinctive character of the roentgen-ray picture (Baumgartner and Abbott [3]). The case here presented illustrates these anomalies in association with inflammatory and degenerative lesions in the pulmonary artery and in its branches. One of the chief interests of this study lies in considering the nature of that arteritis and sclerosis, its etiology and pathogenesis, and the possible mutual relations and effects, both beneficial and deleterious, of the cardiac and arterial lesions on each other.

REPORT OF CASE

Clinical History: R. A., a white male painter, aged 60 years, had always been in good health and a hard worker. For 30 years he drank a pint or more of whiskey daily and led an otherwise dissipated life. He recalled measles and

* Received for publication March 30, 1933.

mumps in childhood but denied syphilis, gonorrhea, scarlet fever, diphtheria, pneumonia and influenza. There was a negative history regarding cyanosis, arthritis, respiratory embarrassments and infections. He was the father of 7 children, 4 of whom died in infancy. His wife had had no miscarriages. Early in the summer of 1931 his present illness began rather suddenly with dyspnea, weakness and fatigue. He stopped work for a few months and took a complete rest in bed.

In November he resumed his occupation and for a time got along fairly well. In December the symptoms returned, aggravated by failing vision, urinary retention, edema, cyanosis, thoracic pain and a persistent productive cough with small hemoptysis on several occasions. In January 1932, for the first time, he consulted a physician. Despite rest and treatment he declined rapidly and progressively and on March 13, 1932 was admitted in a critical condition to the Jefferson Hospital.

Physical Examination: The patient was orthopneic, the skin and mucous membranes deeply cyanosed, and the subcutaneous tissue markedly edematous. The pupils were equal but reacted poorly to light and accommodation. The nails were not curved and the fingers and toes were not clubbed. The temperature was subnormal.

Cardiovascular System: The pulse was 104, regular, full and hard. The jugulars were markedly distended, pulsations being visible in the episternal notch and over the carotids. There was no precordial bulging, the area of cardiac dullness was obliterated, the apex beat was neither visible nor palpable and there was no shock or thrill. The second pulmonic and both aortic sounds were accentuated and a soft blowing murmur was heard over the apex. The blood pressure was 180/120.

Respiratory System: The chest was of the emphysematous type, respirations 26 to the minute, harsh and labored but regular. Fremitus slightly decreased. The percussion note was impaired over both apices and posteriorly over both bases, elsewhere it was hyperresonant. Breath sounds harsh, expiration prolonged. Sibilant and sonorous râles were heard over the entire chest and crepitant râles over both bases and in both axillae.

Abdomen: The spleen was palpable. The edge of the liver was 10 cm. below the last rib.

Morphine and atropine were given, and venesection was performed, 600 cc. of black, thick, viscid, slowly flowing blood being withdrawn. Following this the patient appeared relieved and went to sleep. Six hours later, without any evident clinical change, he emitted one loud gasp and died.

Postmortem Examination

The autopsy was performed 7 hours postmortem. The skin and mucous membranes were a deep purplish red in color, the lips dry and swollen. The subcutaneous tissue of the abdominal wall, genitals, lower extremities, the peritoneum and the wall of the bowel were markedly edematous. The peritoneal cavity contained a slight excess of fluid and the pericardial cavity approximately 300 cc. of clear, straw-colored fluid. The pleural cavities were without adhesions or fluid.

*Heart** (*Fig. 1–A and 1–B*): The organ was rotated clockwise, the pulmonary artery was somewhat horizontal, the walls of all the chambers were hypertrophied and the left ventricle and both auricles were dilated. The right ventricle was in a condition of simple hypertrophy and the chamber was not much enlarged. It extended downward to within 1.5 cm. of the apex, which was formed entirely by the left ventricle. The musculature contained numerous, firm, grayish yellow streaks and flecks. In the upper part of the ventricular septum there was a large, funnel-shaped defect bounded anteriorly and inferiorly by the thickened muscle of the septum, superiorly by the right aortic cusp and posteriorly by the pars membranacea. It tapered from left to right and opened into the sinus of the right ventricle beneath the septal cusp of the tricuspid valve, which stretched across and screened it much as the infundibular cusp stretches across the pulmonary conus in the normal heart. The septal cusp sent two bundles of chordae tendineae from its ventricular aspect to be inserted into the muscular border of the defect about its middle, and the anterior of these contained three large chordae attached to a small papillary muscle. The wall of the conus was hypertrophied and the lumen somewhat narrowed. The latter appeared to have resulted from the compensatory hypertrophy of the crista supra ventricularis, which formed the right anterior margin of the defect on the side of the right ventricle and screened the opening of the defect from the interior of the conus which opened into a dilated pulmonary artery. The endocardium lining the conus and the defect was thickened and opaque. There were no vegetations about the defect or on the tricuspid leaflets. The pulmonary cusps were irregularly widened, the commissures were broadened and the free margins rolled inward. There were several massive aggregations of calcified material on the anterior and right posterior pulmonary cusps, the left posterior segment being free of calcification. The nodule on the anterior segment occupied the right angle of the sinus of Valsalva and extended also on the ventricular surface of the cusp at that point. It was the size of a bean and consisted of an aggregate of small calcified nodules massed together. The right posterior cusp was thickened along its free mar-

* The following gross description of the heart was corrected and revised by Dr. M. E. Abbott, under whose direction the drawings represented in Figs. 1–A and 1–B were prepared. The heart specimen and microscopic slides of all the organs are filed in the Medical Museum of McGill University, Montreal, Canada, Accession No. E–10589.

gin and had two areas of calcification, one nodular and the other along its line of attachment. Above the cusps the pulmonary artery was dilated, inelastic, finely scarred and irregularly thickened by discrete and confluent atheromatous plaques. The mitral leaflets were atheromatous. The aorta was shifted to the right and overrode the ventricular septal defect so that it appeared to arise from both ventricles. It presented several patches of atheroma and calcification, the largest being 1.2 by 0.7 cm. in size. The coronary arteries appeared to be normal. The foramen ovale was closed and the ductus arteriosus obliterated. The coronary sinus was dilated.

Weights and Measurements: Heart 600 gm., 13 by 13 by 8 cm.; mitral valve 10.5 cm. in circumference; tricuspid valve 12 cm.; wall of conus 3 cm. in thickness; walls of right and left ventricles and of the interventricular septum bordering inferior aspect of ventricular septal defect each measured approximately 2 cm. in thickness; dimensions of left ventricular aspect of ventricular septal defect 1 by 2.5 cm.; circumference of lumen of conus at narrowest point 2.5 cm.; circumference of pulmonary orifice at level of cusps 8 cm., and 2 cm. above this line the circumference was 9 cm.; circumference of aorta at semilunar valves and also 2 cm. above this line 7.5 cm.

Lungs: The pleura was slightly thickened, dark red and wrinkled, and beneath it were many red infarcts, between which the lung tissue was tough and fibrous. The bronchi were thickened and their lumens filled with blood-tinged mucus. The lumens of the pulmonary arteries of the first and second order were partially or completely occluded by red thrombi that were propagated into their subdivisions; otherwise, they presented the same changes noted in the main trunk. The smaller intrapulmonary branches were rigid, concentrically or eccentrically thickened, stood out prominently on the cut surface of the lung and spurted blood when incised.

Weights and Measurements: Left lung 630 gm., 25 by 14 by 8 cm.; right lung 770 gm., 28 by 19 by 7 cm.

Anatomical Diagnoses: Ventricular septal defect, dextroposition of the aorta, dilatation of the pulmonary artery, hypertrophy of the walls of the conus and of both ventricles, pulmonary artery sclerosis and arteritis with thrombus formation, healed calcified endocarditis of the pulmonary semilunar cusps, multiple pulmonary infarcts, generalized passive congestion with edema.

Microscopic Examination

In addition to the usual sections pieces of tissue were taken from the pulmonary artery and from several of its branches; from the main bronchi, and from the apices, bases, anterior, posterior and central portions of all lobes of both lungs. They were fixed in dilute formalin and Zenker's fluid, embedded in paraffin and cut and stained with hematoxylin and eosin, Verhoeff's elastic tissue stain, Mallory's and Van Gieson's connective tissue stains, Weigert's fibrin stain, Gram's stain for bacteria in tissues and Levaditi's stain for spirochetes.

Many of the extrapulmonary and large intrapulmonary branches are thrombosed (Fig. 2). The intima is retracted by underlying scars, irregularly destroyed and much thickened by atheromatous, calcified, fibrous and hyalinized plaques that penetrate deeply to replace large portions of the media. The internal elastica is split, frayed, reduplicated and in places much reduced. The intima contains a few small plaques consisting of necrotic cells with distorted and elongated nuclei intersprinkled with lymphocytes, granular leukocytes and multinucleated cells. In the adventitia there are nodular and linear collections of lymphocytes, plasma cells and proliferating fibroblasts about the vasa vasorum, which are markedly thickened (Fig. 3). This inflammatory tissue projects fanwise into the media to form stellate scars that interrupt and replace the muscularis and elastica (Figs. 4 and 5). A somewhat similar inflammatory process is observed in the tissue surrounding the arteries. The walls of the intrapulmonary arteries measure 1 to 3 mm. in diameter (Fig. 6) and are devoid of inflammatory cells, the vasa vasorum are thickened, and the muscularis of the media is largely replaced by an increase in fine and coarse collagen and distorted elastic fibrils. The internal elastica is hypertrophied, split, frayed and reduplicated. The lumens are partially occluded by fresh and organized thrombi. Atheroma and calcification are rarely encountered. The perivascular granulation tissue is prominent. The intrapulmonary arteries below 0.5 mm. (Fig. 7) seem fewer in number but proportionately thicker than normal, and the muscularis is largely replaced by connective tissue. The internal and external elastica are distorted and occasionally fused. There are fresh, organized and canalized thrombi, and hyalinized papillary nodules project into the lumen. Most of

the walls are free of inflammatory cells but a few are diffusely infiltrated by lymphocytes and endothelial leukocytes. With the decrease in the size of the arteries, the perivascular granulation tissue becomes correspondingly more prominent. It varies from active inflammatory nodules to dense hyaline, linear and stellate scars that stretch at length through the pulmonary parenchyma. The capillaries and small veins appear dilated, reduced in number, thickened and hyalinized. Some of the larger veins are thrombosed. The pulmonary alveoli vary in size, many are enlarged and their walls thin and ruptured. In other areas the septa are fibrosed and thickened, and contain interstitial vascular nodules consisting of lymphocytes, plasma cells and fibroblasts that invade and obliterate the air spaces (Fig. 8). Infarcts are numerous, hemorrhage extensive, heart-failure cells plentiful. Anthracosis is minimal. The epithelium of the bronchi is hyperplastic and in many places denuded. The walls are thickened by vascular collections of lymphocytes and fibroblasts which occasionally penetrate through the wall to invade the lumens. There is a subintimal thickening of the bronchial arteries and a chronic proliferative reaction in the peribronchial tissue. The pleura is diffusely thickened by hyalinized and cellular connective tissue containing small, discrete, vascular lymphocytic foci. The aorta contains degenerative changes of the nature of atherosclerosis, the media is somewhat hypoplastic and the vasa vasorum are slightly thickened. There are submiliary nodules consisting of sparse infiltrations of lymphocytes, plasma cells and endothelial leukocytes about a few newly formed capillaries in the media. In the myocardium are many irregular, loose, nodular, cellular collections consisting mainly of endothelial leukocytes, proliferating fibroblasts and necrotic muscle fibers. Intermediate stages of healing can be traced up to dense hyalinized scars that frequently contain one or more blood vessels in the center.

Spirochetes or other organisms are not demonstrated in any of the tissues.

Discussion

The Cardiac Defect: With the combination of ventricular septal defect and dextroposition of the aorta, without associated pulmonary stenosis, the average life is 16 years; the highest age attained heretofore is 33 years. The patient whose case is under discussion lived to

be 60. Because of his critical condition the physical examination was necessarily superficial. He was in the hospital only 8 hours when death occurred, so that careful studies were not carried out and a positive ante mortem diagnosis was not made. As the anatomical conditions in the heart favored oxygenation, the admixture of venous with arterial blood was negligible, which accounts for the absence of clubbing and until the last illness the absence of dyspnea and cyanosis. The relatively high pressure in the left ventricle, the healed pulmonary valvulitis, the contour of the ventricular septal defect, the patches of fibrosis on the endocardium bordering the defect and on the wall of the conus opposite support this assumption. The ventricular septal defect probably actually contributed to longevity by permitting the force of both ventricles to combine in propelling blood through the lesser circuit against the resistance offered by the sclerosed pulmonary arteries. Dyspnea and cyanosis were of course coincidental with the onset of myocardial insufficiency, when in all probability the shunt through the defect became venous-arterial. It has been considered in these cases that the pulmonary artery undergoes dilatation to accommodate the additional volume of blood received from the left ventricle through the defect into the pulmonary circulation. In our case it is quite possible that it was due in part to the arteritis (Warthin [4]). In Blechmann and Paulin's case,[5] the dilatation was attributed to a streptococcic pulmonary endarteritis.

An inflammatory arteritis of the pulmonary arteries and arterioles complicating congenital heart disease is exceedingly rare (Abbott [2]). The nature of the lesions in our case rests mainly on their anatomical and histological appearances and involves a consideration of syphilis, atherosclerosis, hyperplastic arteriosclerosis and rheumatic arteritis.

The characteristics of syphilitic pulmonary artery sclerosis and arteritis have been well established (Cheney,[6] Hare and Ross,[7] Konstam and Turnbull,[8] Karsner *) by the occurrence of pulmonary arterial aneurysms and the demonstration of spirochetes in the walls (Warthin and coworkers,[9, 10]); by significant lesions of the pleura,

* Since submitting this paper for publication the following article has come to our attention.

Karsner, H. T. Productive-cicatricial syphilitic disease of the pulmonary artery. *Arch. Int. Med.*, 1933, 51, 367–386.

bronchi and pulmonary parenchyma (Landsberg,[11] McIntyre[12]); positive Wassermann and Kahn reactions on the blood serum of patients (Winternitz and Schmeisser[13]), and the finding as associated conditions paresis, tabes, aortitis, chronic orchitis and visceral gummas. Although the evidence in our case is incomplete from the clinical aspect, the prevailing character of the inflammatory reaction simulates more that of syphilis than of any other type of lesion. It is possible that all the lesions may represent the result of the fetal syphilis that ranks high as an etiological factor in congenital heart disease. This is strengthened by the character of the myocardial lesions (Abbott[2]). Some authors (Warthin[14] and Boyd[15]) have described a syphilitic type of myocarditis that they regard as being quite characteristic of the disease. We are not in accord with their opinion but agree with Saphir,[16] who believes that no myocardial lesion can be used as a diagnostic criterion of acquired syphilis. In a rather extensive search of the literature we have encountered no proved case of syphilitic pulmonary arteritis complicating congenital heart disease. Arrillaga's speculations in this connection[17] are not well supported and await confirmation.

A degenerative atherosclerosis develops in the larger branches of the pulmonary arteries secondary to a variety of congenital and acquired lesions of the heart, lungs, pericardium, pleura and blood vessels (Miller[18]). It is not uncommonly observed in association with defects at the upper part of the interauricular septum, in which case such a large influx of blood is sent into the pulmonary circulation that it creates a strain on the arterial walls. It is rarely seen with the type of defect dealt with here and never to so marked a degree (Abbott*). We consider that it was probably engrafted on the inflammatory process mainly as a secondary manifestation, which is not uncommon in syphilitic aortitis. The chronic alcoholism and possible lead poisoning (see occupation) might have contributed to it (Karsner[19]), although the opinion that lead can cause arteriosclerosis is founded on autopsy material in lead workers (Aub and coworkers[20]) and is without experimental support (Ophüls[21]).

A condition of chronic cyanosis, erythrocytosis, and dyspnea occasionally results from hyperplastic sclerosis of the finer pulmonary arterial radicles. Histologically it is characterized by proliferation and reduplication of the internal elastic lamina and a

* Personal communication.

corresponding atrophy and fibrosis of the media (Bacon and Apfelbach [22] and Rosenthal [23]). The pleura, bronchi, pulmonary parenchyma and walls of the pulmonary arteries are devoid of inflammatory cells. Such a case has recently come under our observation (Sokoloff and Stewart [24]) and except for a few of the changes in the smaller arteries we find the lesion entirely unlike the one dealt with in the present communication.

A more or less characteristic inflammatory reaction, varying somewhat with the stage of the disease (Swift [25]) occurs in the pulmonary artery (Paul,[26] Kugel and Epstein [27]), pulmonary parenchyma (Rabinowitz,[28] Naish,[29] Sacks,[30] Eiman and Gouley [31]), pleura (Paul [32]), and bronchi (Fraser [33]) in certain persons suffering from rheumatic fever. The changes affect the smaller pulmonary arterial twigs and consist of fibrin, erythrocytes and multinucleated cells in the muscularis and immediately beneath the endothelium. The internal elastic lamina becomes frayed and permanently lost. Surrounding the affected vessels are fibrin, erythrocytes, granulocytes in great numbers, radially arranged cells with bizarre nuclei, and beyond these a few plasma cells and dilated capillaries. Healing is specific, thrombi are never encountered and the extrapulmonary branches are uninvolved (Pappenheimer and VonGlahn [34, 35]). In our case a few small arteries presented lesions resembling somewhat those representative of the healing stage. However, the prevailing character of the active perivascular, pleural, bronchial and pulmonary parenchymal granulation tissue, the inflammation and extensive thrombosis of the extrapulmonary arteries were more illustrative of syphilis than of rheumatic fever. The viscera were carefully and critically scrutinized for evidence of rheumatic lesions. The nodules on the pulmonary cusps were suggestive, but similar active, non-rheumatic vegetations have been noted before in congenital heart disease (Moschcowitz [36]). The access of arterial blood to the right heart is thought to be in some way the determining factor (Boldero and Bedford [37]). Sections selected from the free margins and bases of the pulmonary cusps and from the musculo-arterial junction failed to reveal rheumatic lesions (Kugel and Epstein [27]). The vast majority of the myocardial lesions were non-specific and may well have been vascular in origin. A few resembled Aschoff nodules which VonGlahn and Wilshusen [38] observed in the myocardium of subjects whose aortas presented characteristic, well advanced lesions of

syphilitic arteritis. The mild inflammatory changes in the aorta of our case were not distinctive and might possibly have been due to rheumatic fever (Pappenheimer and VonGlahn [34, 35]) or to latent syphilis. Hare and Ross [7] found the aorta uninvolved in 8 of 16 unquestioned cases of syphilis of the pulmonary artery.

Summary and Conclusions

The clinical, anatomical and histological features of a case of pulmonary artery sclerosis and arteritis associated with an interventricular septal defect, dextroposition of the aorta and dilatation of the pulmonary artery are presented and discussed. There were no symptoms until the last few months of life and death occurred at the age of 60 years, the highest ever attained by an individual with this combination of congenital lesions.

The shunt through the defect in the ventricular septum was probably arterio-venous; this permitted the combined force of both ventricles to unite in overcoming the resistance offered by the sclerotic lesser circuit.

The evidence adduced, although not empirical, strongly favors a syphilitic origin for the lesions in the pulmonary artery, the pulmonary parenchyma, the pleura and bronchi.

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DESCRIPTION OF PLATES

PLATE 105

FIG. 1–A. Anterior view of the heart illustrating marked hypertrophy of the crista supraventricularis (beneath which the defect opens) and of the wall of the right ventricle and conus, the lumen of which is narrowed. Note the dilatation, scarring and sclerosis of the pulmonary artery, the thickening of the cusps and the widening of the commissures.

FIG. 1–B. Posterior view of the heart illustrating the large funnel-shaped defect in the upper part of the interventricular septum, just beneath the right aortic cusp. Note the dextroposition and slight atheroma of the aorta. The incised myocardium contains numerous fine, grayish flecks and streaks.

I—B

I—A

PLATE 106

FIG. 2. Large intrapulmonary branch of the pulmonary artery with thrombus formation, perivascular fibrosis and marked destruction of the wall. Photograph of histological slide, 1¾ times actual size. Elastic tissue stain.

FIG. 3. Round cell infiltration of the vasa vasorum of the pulmonary artery extending into the media. The intima is thickened by hyalinized and atheromatous plaques. × 57.

FIG. 4. Wall of the pulmonary artery where the inflammatory changes are similar to those observed in Fig. 3. The involvement of the media is more extensive. × 35.

FIG. 5. Inflammatory reaction in the portion of the media contained within the circle in Fig. 4. Note the leukocytic infiltration, newly formed vessels and fibrosis. × 115.

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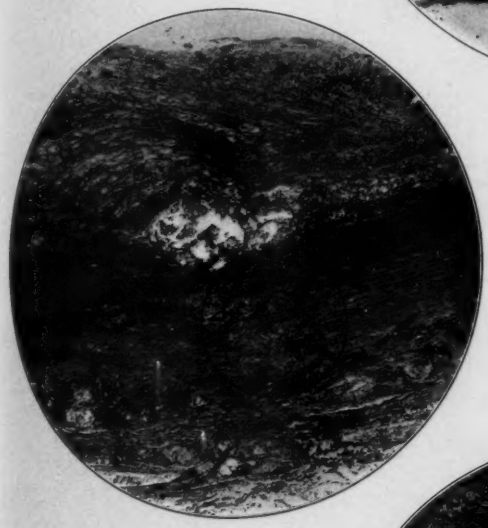

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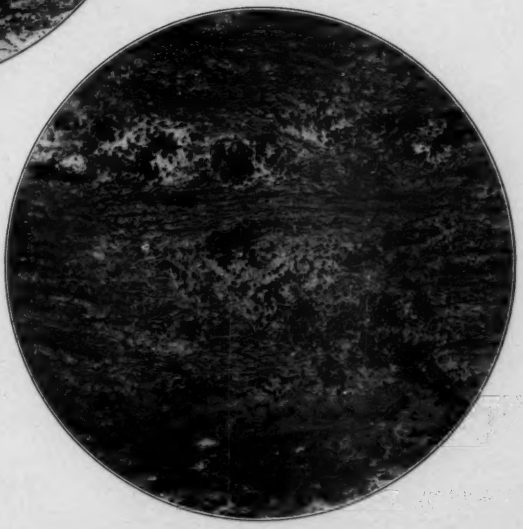

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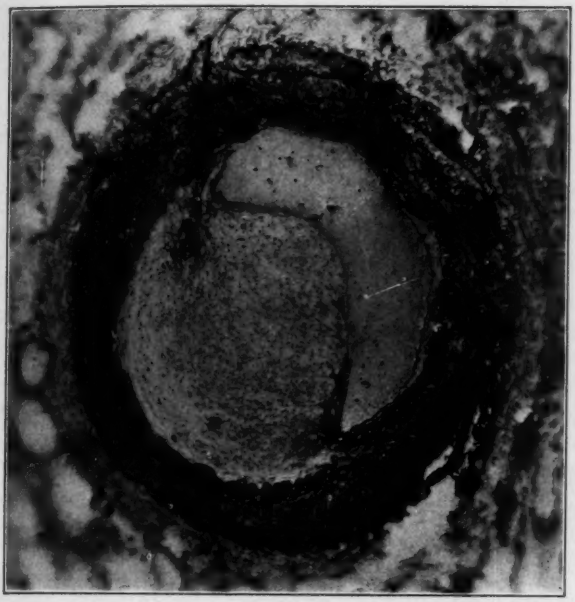
Congenital Heart Disease with Pulmonary Arteritis

PLATE 107

FIG 6. A branch of the pulmonary artery illustrating detailed changes in elastic fibers; the lumen is partially occluded by an organized thrombus. Elastic tissue stain. × 85.

FIG. 7. A small branch of the pulmonary artery with marked obliterative endarteritis and perivascular fibrosis. × 130.

FIG. 8. An interstitial pulmonary inflammatory nodule forming an eccentric collar about a small thickened branch of the pulmonary artery. The nodule is markedly vascular and consists of lymphocytes, plasma cells, endothelial leukocytes and proliferating fibroblasts. × 100.



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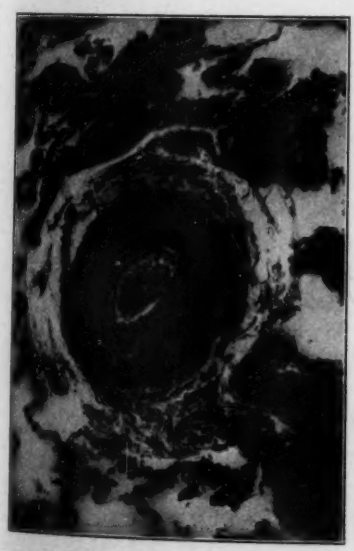

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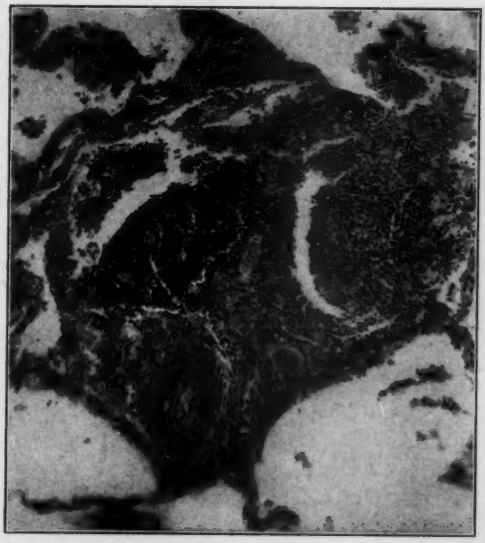

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Congenital Heart Disease with Pulmonary Arteritis

A REDUCING SUBSTANCE FOUND IN CHROMOPHILIC ADENOMAS AND IN THE NORMAL ANTERIOR PITUITARY*

TRACY J. PUTNAM, M.D., AND HERBERT B. WILCOX, A.B.

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The present investigation was prompted by a chance observation. When examining an operative specimen from a patient with active acromegaly a smear of the tumor (a typical acidophilic adenoma) was stained with methylene blue. The cells were deeply stained and their histological characteristics well displayed at first, but while the slide was being examined all traces of blue color rapidly disappeared. Other smears were stained and the same slide restained, with always the same result: the smallest fragment of tumor tissue was able to decolorize many times its volume of approximately 1 per cent methylene blue within fifteen minutes. Each time the color returned on exposure to air. Unfortunately we have not had further opportunity to confirm this single observation.

Although many surviving tissues have the property of reducing methylene blue, none that we have encountered can compare with this particular tumor tissue in its activity. A chromophobe adenoma of the anterior lobe offered the most interesting comparison. Smears from fresh tissue removed at operation in precisely the same way retained color imparted to them by the same solution for over twenty-four hours.

Fresh pieces of normal beef anterior pituitary reduce methylene blue much more rapidly than do neighboring structures, a fact noticed by Dandy [1] when he attempted a vital staining of the gland *in situ*. But the reducing power of anterior lobe tissue is not strikingly greater than that of liver, muscle and certain other tissues that have been studied so thoroughly by Thunberg [2] and his followers.

Further investigation showed, however, that the mechanism of reduction is quite different from that of the hydrogen transportase system studied by Thunberg. In the first place the reaction is proportionately accelerated by successive increases in temperature up to 100° C, and this fact indicates that the substance involved is

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probably an actual reducing agent in itself, rather than an enzyme-substrate system. It does not, however, reduce Benedict's solution or give the other common tests for sugar.

In the second place the reducing substance may be extracted from fresh glands by weakly alkaline solutions and, if filtered or centrifuged off, may be kept for weeks in the cold as an opalescent protein solution. If the protein is precipitated by the ordinary methods (sodium sulphate, alcohol, acidification) most but not necessarily all of the reducing substance is carried down with it.

The question naturally at once arises, is the reducing substance related to any of the physiologically active principles of the anterior hypophysis? The characteristic constituent, and probably the quantitatively predominant one, in the acidophilic adenoma is the growth-promoting principle, while any constituents that such a tumor may have in common with a chromophobe adenoma may apparently be ruled out as a source of the reducing substance. The growth-promoting principle derived from normal anterior lobes clings stubbornly to the globulin fraction, and so does the reducing substance. On the other hand, the reducing substance survives many procedures (such as boiling) that are known to be fatal to the growth principle. Tests on immature female rats show that the reducing substance is not identical with the estrus-producing substance.

Further studies on methods of quantitating the reducing substance on its chemical nature and on its relation to the growth principle are being carried on by Dr. William Salter and Dr. Arda Green.

Summary

A substance reducing methylene blue has been demonstrated in the normal anterior pituitary and its extract, and in larger amounts in a chromophile adenoma. None was found in a chromophobe adenoma.

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THE EFFECT OF TESTICULAR EXTRACT ON EXPERIMENTAL TUBERCULOSIS IN RABBITS. I. SKIN LESIONS *

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Duran-Reynals [1, 2, 3] has shown that there exists in the testes of normal rabbits a substance that has the remarkable power of enhancing skin infections produced by vaccine virus and by staphylococci. Hoffman [4] confirmed the observation on the enhancement of vaccine virus and extended the work to show that this phenomenon is equally effective with the viruses of vesicular stomatitis, Borna disease, and herpes; and in the case of the viruses of herpes, Borna disease, and vaccinia, the enhancement applied also to central nervous system infections. Pijoan [5] reported that this substance, when added to cultures of twenty different bacteria, caused enhancement to a high degree of the resulting intradermal lesions. Hoffman and Duran-Reynals [6] found that the Berkefeld filtrate of testicular extract was equally as effective as the unfiltered emulsion. To this enhancing substance Ledingham and Barratt [7] have given the name "Reynals factor."

Since all the bacteria used in the above mentioned experiments were those that usually produce a more or less uniform, acute reaction, it was felt that it might be of interest to apply this enhancing substance to an organism giving an entirely different type of reaction, *i.e.*, to the tubercle bacillus. Consequently experiments were designed to determine what effect, if any, testicular extract might have upon the character of the lesions resulting from the intradermal injection of tubercle bacilli in rabbits.

METHODS AND MATERIALS

The tubercle bacilli used in these experiments consisted of avian, human, and bovine strains. The organisms were grown on a modified egg medium † and were from 1 to 2 months old at the time of

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† The modified egg medium is prepared as follows:

Potatoes	250 gm.
Distilled water	500 cc.
Glycerin	20 cc.

Autoclave 30 minutes under 15 lb. pressure.

To 50 cc. of the above potato glycerin extract add 50 cc. bouillon and 5 eggs. Put in tubes and inspissate. Add 1 cc. distilled water to each tube.

Autoclave 20 minutes under 15 lb. pressure.

use. A heavy, fresh saline emulsion of the organisms was used for inoculation.

Testicular extract was freshly prepared from testes of healthy adult rabbits as follows. The testes were removed aseptically,* and after the fat was dissected away they were weighed, minced and ground with sand in a sterile mortar. Sufficient Locke's solution was added to make a 1:10 suspension and the mass was then centrifuged at high speed for 15 minutes. The supernatant fluid was passed through a Berkefeld Y filter. This filtrate constituted the extract used in the experiments and it was tested for sterility before use.

Injection masses for each of the avian, human, and bovine strains were prepared for inoculation as follows. The saline suspension of the organisms was divided into two equal parts. To the first part was added an equal volume of testicular extract, and to the second, an equal volume of sterile normal saline. Thus the first mass served as the experimental material and the second as its control. For each strain of organism the injection masses contained approximately the same number of organisms and differed from each other only in that the experimental mass contained testicular extract, whereas the control did not.

Nine healthy, young adult rabbits were used in the experiments. The flanks were clipped and then shaved. The animals were divided into three groups of three animals to each group. One group was used for the avian, one for the human, and one for the bovine strains.

For the experiment in which the avian strain was employed 1 cc. of the experimental injection mass containing a saline suspension of organisms plus an equal volume of testicular extract was injected intradermally in one area, and 1 cc. of the corresponding control was injected in like manner in another part of the shaved skin on the same side. Two animals were thus inoculated. The third received similar injections, with the exception that both sides of the animal were inoculated.

The three animals receiving the bovine strain were inoculated in similar fashion with two control and two experimental injections on either side.

The remaining three rabbits received four inoculations consisting of 1 cc. of the testicular extract-bacilli suspension (human strain)

* All operations on animals were carried out under full ether anesthesia.

on each side, and likewise four inoculations of saline-bacilli emulsion. Thus each animal received a total of sixteen inoculations. The animals were kept in cages and fed the usual laboratory diet.

Twelve to eighteen days were allowed for the disease to develop, during which time the size and character of the lesions were noted from day to day. The animals were then killed and tissue from both the experimental and the control lesions was removed and fixed in a mixture of alcohol and formalin (9 parts alcohol and 1 part formalin). Celloidin sections were cut and stained by the carbol fuchsin method of Ziehl-Neelson.

It was noted at the time of the inoculations that all injections containing testicular extract diffused so rapidly that no wheal was visible at the end of 2 minutes, whereas those injections without testicular extract resulted in wheals that were visible as long as 30 minutes after inoculation.

Results

The lesions resulting from the injection of organisms with testicular extract were without exception more severe and much more extensive than the respective control lesions. This difference was found to be constant, irrespective of the type of organism used. All early lesions in every series were similar in appearance, but as the disease progressed, those resulting from the inoculation of bacilli with testicular extract became more extensive, showed necrosis and ulcerated earlier than the corresponding control lesions.

The lesions resulting from the inoculation of avian or bovine bacilli were more extensive and more severe than those resulting from the injection of the human strain in both the control and experimental injections.

The microscopic changes consisted of a marked infiltration with lymphocytes and monocytes, with occasional tubercle formation. There were also varying degrees of necrosis in some instances. Acid-fast bacilli (occurring singly or in clumps) were present in all lesions. There was no essential qualitative microscopic difference in either the avian, human or bovine lesions and their respective controls.

In order to express the enhancement phenomenon in more concrete terms the average plane area of the lesions in every series was computed. Those due to the avian bacilli plus saline averaged

TABLE I

Avian Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
	cc.	*cc.*	*days*	*cm.*	*cm.*
A	1	1	12	3 x 3	15 x 4 Marked ulceration
B	1	1	12	4 x 4 Ulcerated	12 x 6 Marked ulceration
C	1	1	13	3 x 2.5	6 x 3.5
C	1	1	13	3 x 2	6 x 3 Ulcerated

TABLE II

Bovine Strain

Rabbit	Inoculation		Duration	Size and character of lesions	
	Equal parts emulsion plus saline	Equal parts emulsion plus testicular extract		Emulsion plus saline	Emulsion plus testicular extract
	cc.	*cc.*	*days*	*cm.*	*cm.*
D	1	1	15	3 x 3 Ulcerated	12 x 6 Marked ulceration
D	1	1	15	3 x 2.5 Ulcerated	8 x 3.5 Ulcerated
E	1	1	16	3 x 1.5	4.5 x 4 Moderate ulceration
E	1	1	16	2.5 x 1.5 Slight ulceration	5 x 3 Moderate ulceration
F	1	1	16	1.5 x 1.5	4.5 x 4 Slight ulceration
F	1	1	16	1.5 x 1	4 x 2.5 Slight ulceration

9.6 sq. cm. in diameter, whereas those following injection of the bacilli-testicular extract mass averaged 42.7 sq. cm. Thus, the latter lesions were 4.3 times the size of their controls. With the bovine

TABLE III

Human Strain

Rabbit	Site for injection	Inoculation				Duration	Size and character of lesions	
		Equal parts emulsion plus saline		Equal parts emulsion plus testicular extract			Emulsion plus saline	Emulsion plus testicular extract
		No.	*Amt. in cc.*	*No.*	*Amt. in cc.*	*days*	*cm.*	*cm.*
G ...	Right side	4	1	4	1	14	Separate nodules about 1.3 x 1.3 Slightly ulcerated	Confluent lesion 9 x 3.5 Moderate ulceration
G ...	Left side	4	1	4	1	14	Separate nodules about 1.2 x 1.2 Slight ulceration	Confluent in denoted lesion 9.5 x 3.2 Moderate ulceration
H ...	Right side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 3.5 Marked ulceration
H ...	Left side	4	1	4	1	14	Separate nodules about 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9 x 4 Marked ulceration meeting in midline
I ...	Right side	4	1	4	1	18	Separate nodules 1.5 x 1 Slight ulceration in centers	Confluent lesion 8.5 x 3.5 Moderate ulceration
I ...	Left side	4	1	4	1	18	Separate nodules 1.5 x 1.5 Slight ulceration in centers	Confluent lesion 9.2 x 3.4 Moderate ulceration

strain the controls averaged 4.75 sq. cm., while those resulting from testicular extract injection were 28.6 sq. cm. The latter were therefore 5.6 times the controls in size. The lesions due to human bacilli were more difficult to measure with any reasonable degree of accu-

racy, because a large number of inoculations were made in a small space and became confluent. However, as accurately as could be determined, the control lesions averaged 1.82 sq. cm. in area, while the experimental lesions averaged 7.875 sq. cm. The size of the latter was 4.3 times that of the controls.

The results of our experiments are represented in tabular form (see Tables I, II and III).

Discussion

Considerable evidence has been presented in the literature in support of the fact that testicular extract will increase the severity and extent of acute lesions resulting from pyogenic bacteria or viruses.[1-5] Our results clearly demonstrate that testicular extract is equally effective in enhancing the lesions resulting from the intradermal injection of avian, bovine or human strains of tubercle bacilli in rabbits. These organisms normally produce lesions that differ from those previously reported in that the incubation period is longer and the reaction is of an entirely different type. Thus, the enhancing effect of testicular extract in infections appears to be general, whether such infections be acute or chronic, bacterial or viral.

It is well known that the avian and bovine strains are much more virulent for rabbits than the human strain. We have found this same relative difference in the severity of the resulting skin infections due to these organisms. Regardless of the size of the control lesions those resulting from the injection of bacilli together with testicular extract were approximately five times as extensive. From these observations and the work of others, it appears conclusive that the extract of normal rabbit testes possesses some property of enhancing the severity of representative lesions from all classes of infections.

It was noted at the time of inoculations that the injection masses containing testicular extract diffused very rapidly, which is in accord with the observations of Hoffman and Duran-Reynals [6] and of McClean.[8] It is possible that the resulting enhancement of the infections may be due either to some action of the active principle of testicular extract on the organisms or on the host or both. It seems impossible to say on the basis of our observations whether the enhancement is due to a wider distribution of organisms, an increase in virulence, a decreased resistance of the host, or a combination of these factors. The reader is referred to papers by Hoffman, Duran-

Reynals, and McClean for a discussion regarding the mechanism of action of testicular extract.

The results of these experiments suggest the possibility of employing this enhancing factor in a clinical connection in animal inoculation of body fluids and exudates where such a procedure is necessary to make a diagnosis of tuberculosis.

Conclusions

1. When tubercle bacilli together with testicular extract are injected into the skin of rabbits marked enhancement of the resulting lesions occurs.

2. This enhancement takes place equally well with the human, bovine, and avian strains.

3. The practical application of this enhancement phenomenon in laboratory diagnosis is worthy of consideration.

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By vote of the Council of the American Association of Pathologists and Bacteriologists this issue of the *American Journal of Pathology* is dedicated to Frank Burr Mallory in honor of his seventieth birthday and the opening of the Mallory Institute of Pathology of the Boston City Hospital.

The authors of the articles are graduates of the laboratory now in pathology. Since the number of papers was necessarily limited by the space available the contributors were chosen on a chronological basis, with respect to the dates of their service in the laboratory.

The number was planned and edited by the assistant editorial staff.